Chem 452 - Lecture 5
Catalytic Strategies
111026

Enzymes have evolved an array of different strategies or enhancing the power and specificity of the reactions they catalyze. For numerous enzymes the details have been worked out at the atomic level. In this lecture we will focus on four examples: chymotrypsin, carbonic anhydrase, the EcoRV restriction endonuclease, and myosin II ATPases.

Introduction

- Enzymes exhibit both catalytic power and specificity
- We will consider closely, four examples.

Introduction

- Chymotrypsin (1gct) 3.4.21.1
  - A Hydrolase, which cleaves peptide bonds in proteins
- Carbonic anhydrase (1ca2) 4.2.1.1
  - A Lyase, which adds water to CO_2.
- EcoRV (1rvb) 3.1.21.4
  - A Hydrolase, which cleave phosphodiester bonds in DNA
- Myosin motor domain ATPase (1fmv & 1fmw) 3.6.4.1
  - An enzyme that couples the hydrolysis of ATP to the mechanical motion.

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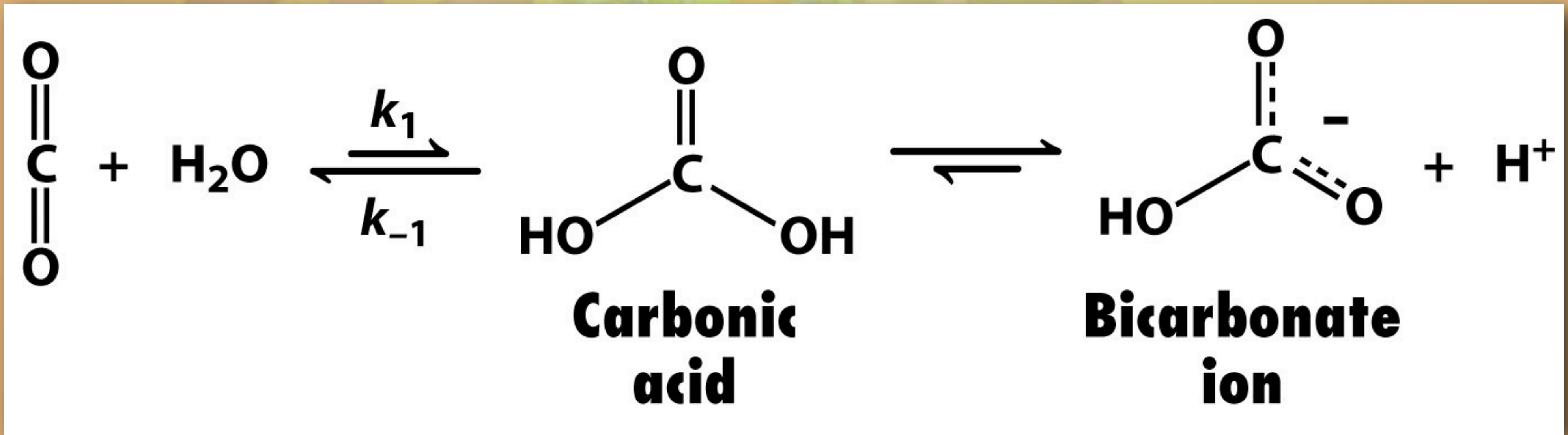
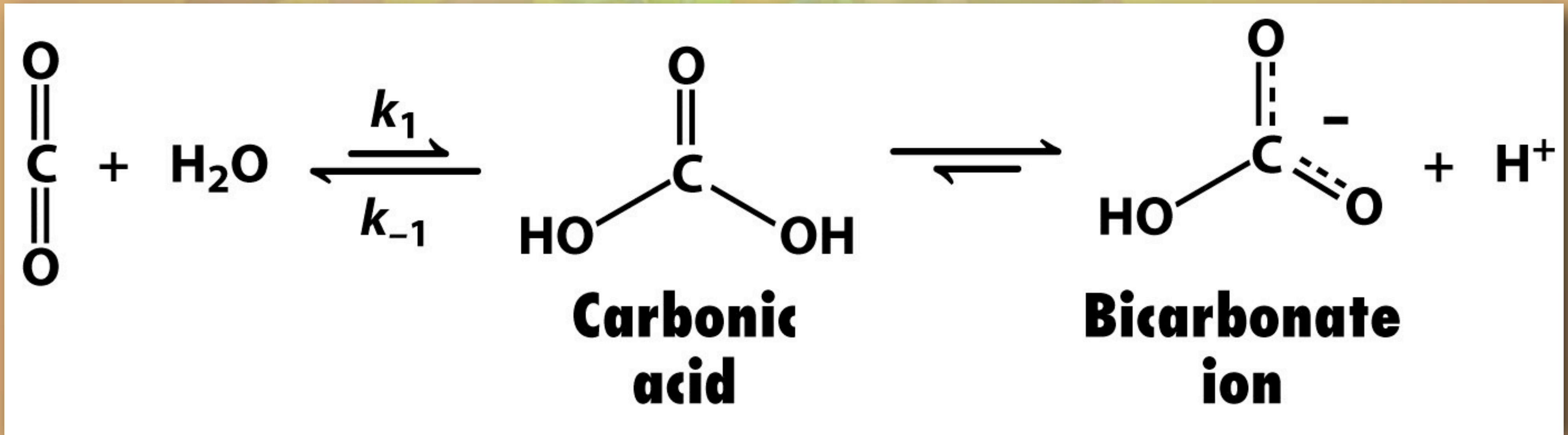
Carbonic Anhydrase

- CO_2 is a major waste produce of the catabolic (energy producing) metabolic pathways.
  - Transported out of the tissues as HCO_3^-.



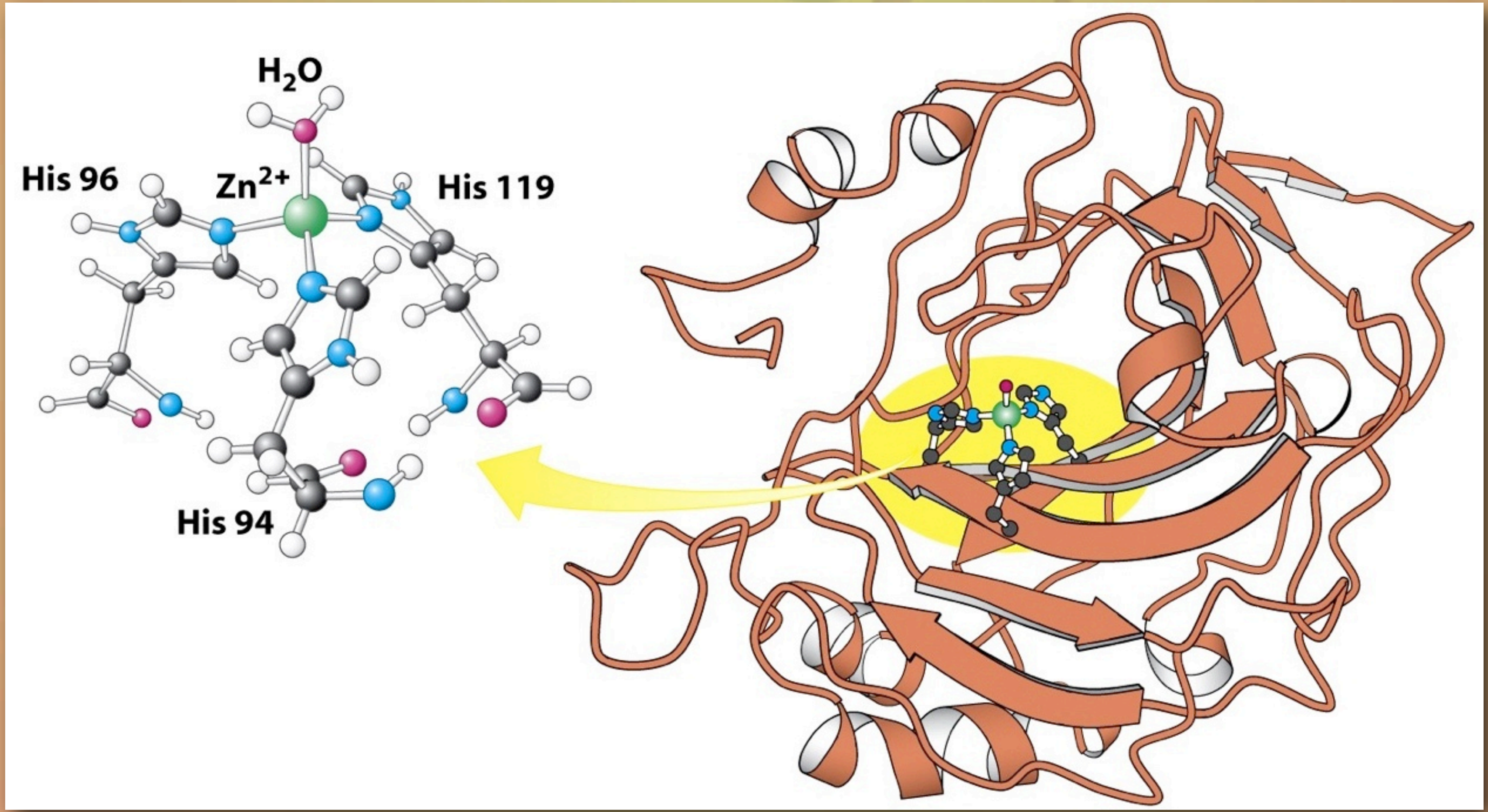
Carbonic Anhydrase

- While the uncatalyzed reaction is overall kinetically favorable, speed is of the essence.
  - Carbonic anhydrase is able to increase the catalytic rate constant to $k_{cat} = 10^6\ s^{-1}$!



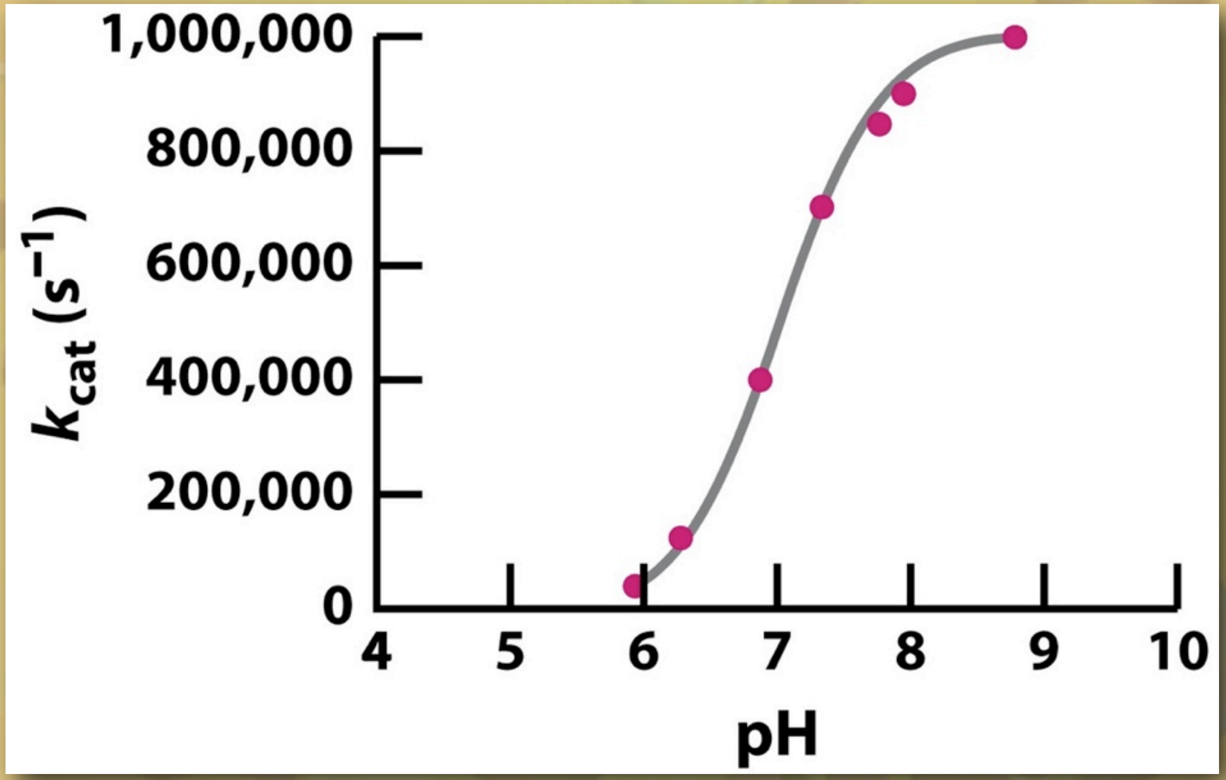
Carbonic Anhydrase

- The nucleophile in this reactions is OH^-
  - A Zn^{2+} ion is involved in generating the nucleophile



Carbonic Anhydrase

- The pH profile reveals a group that is involved in the catalysis, which has a pK_a of around 7



- Studies concluded that this was due to ionization of a water molecule.

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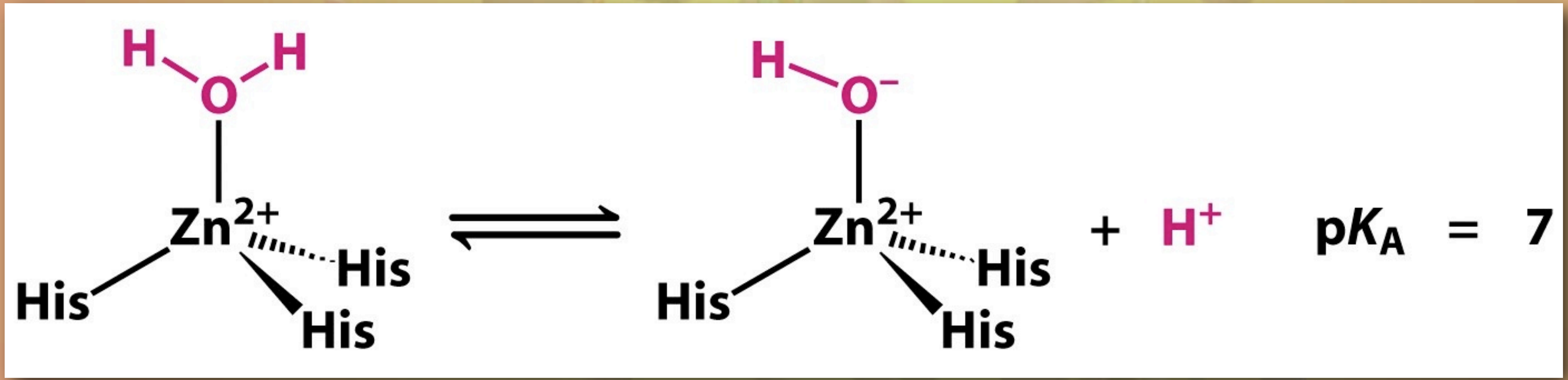

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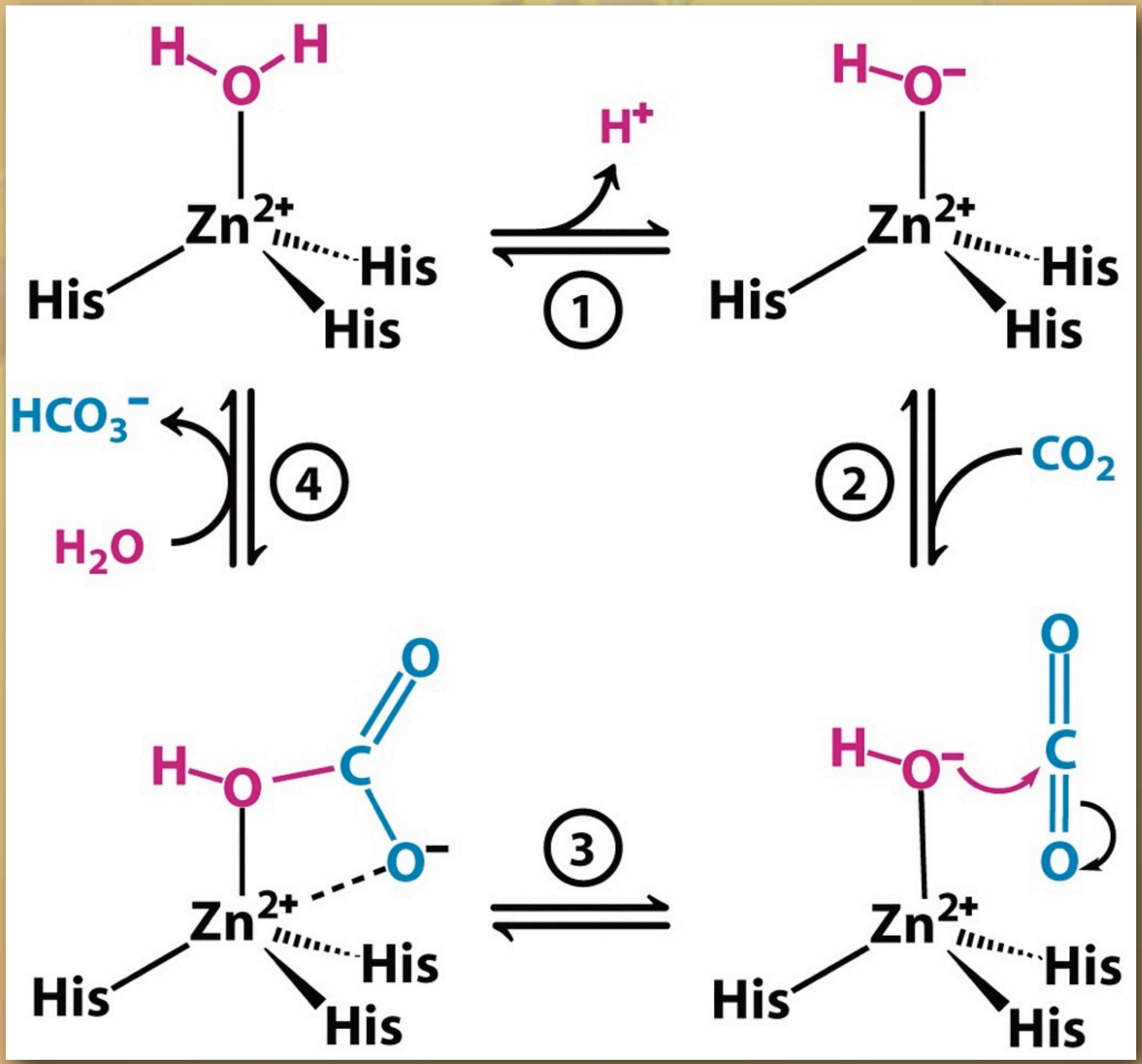
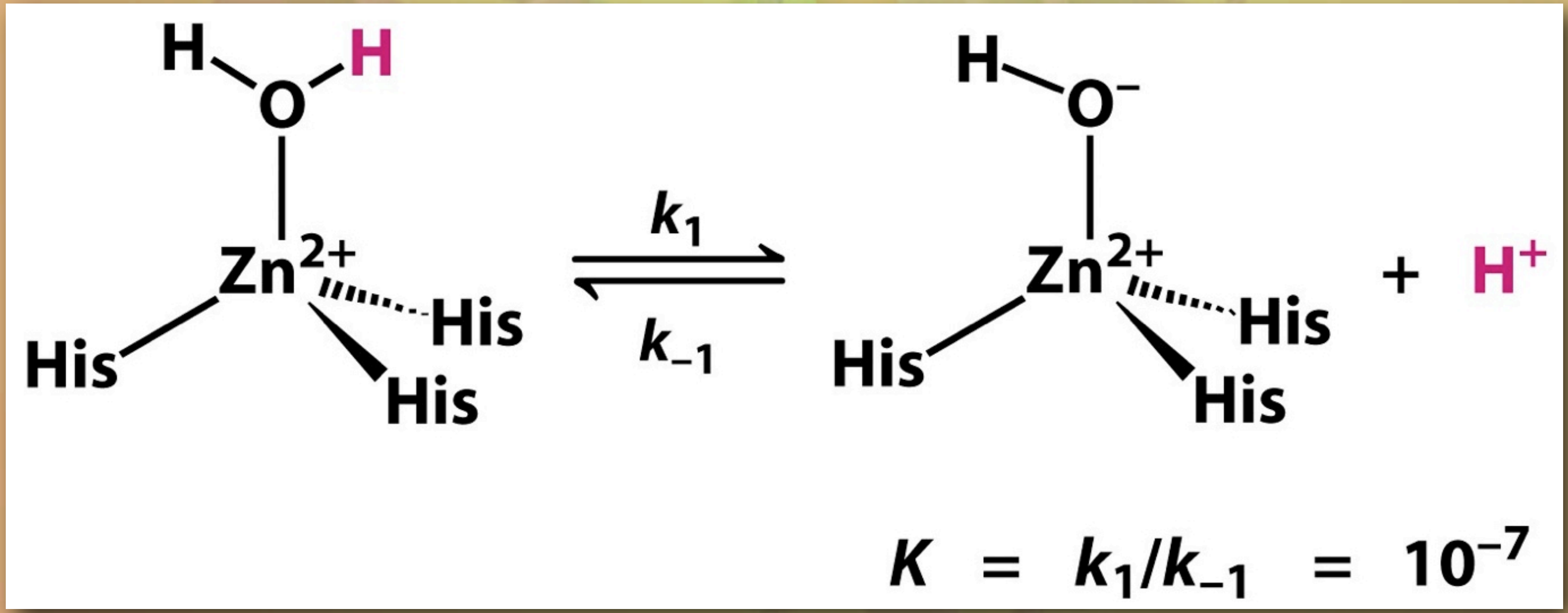
Cabonic Anhydrase

- The catalytic cycle for carbonic anhydrase



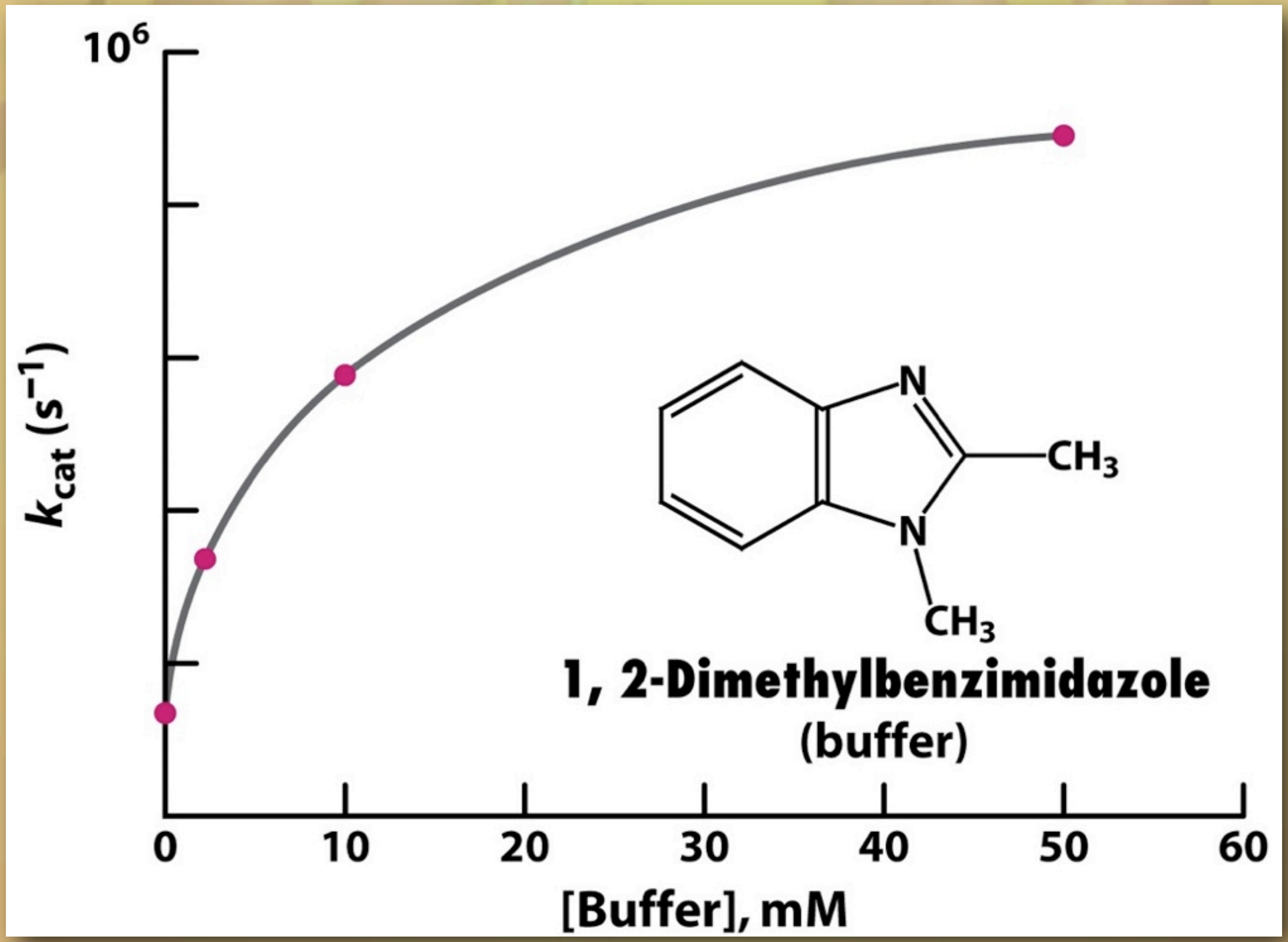
Carbonic Anhydrase

- The catalytic cycle for carbonic anhydrase
  - Because H^+ ions diffuse very rapidly ($k_{-1} \approx 10^{11}$ $M^{-1}s^{-1}$), $k_{cat} = K \bullet k_{-1} \approx 10^4\ s^{-1}$ (not $10^6\ s^{-1}$, as observed)



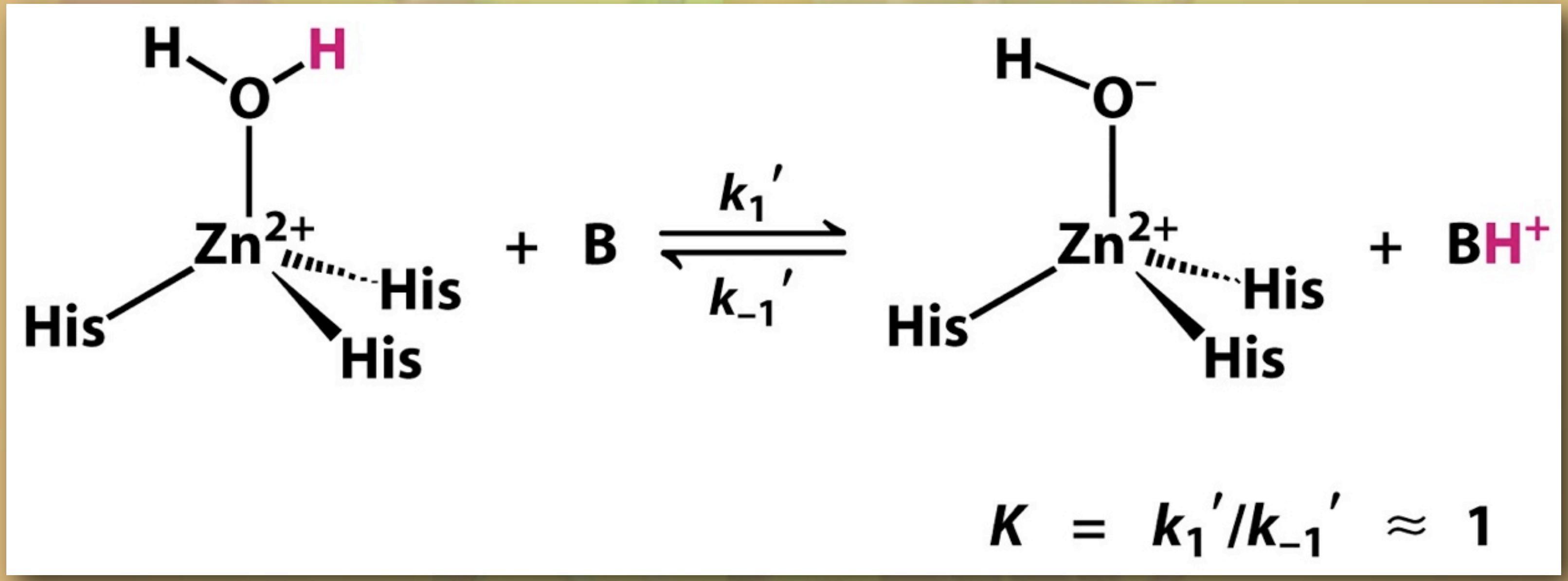
Carbonic Anhydrase

- Buffers can be shown to speed up the carbonic anhydrase reaction.



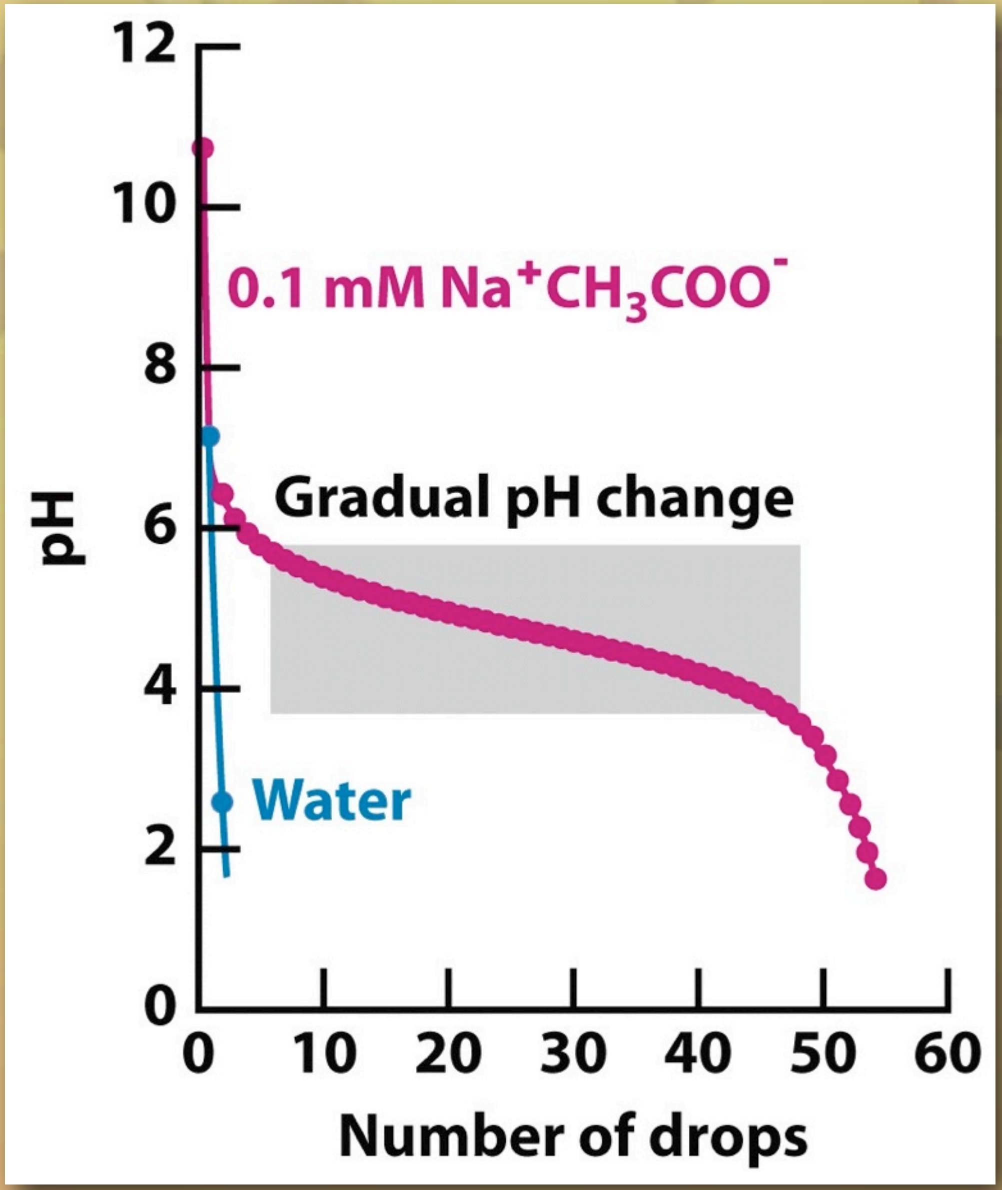
Carbonic Anhydrase

- Buffers can be shown to speed up the carbonic anhydrase reaction.
  - They help shift the reaction to the right.



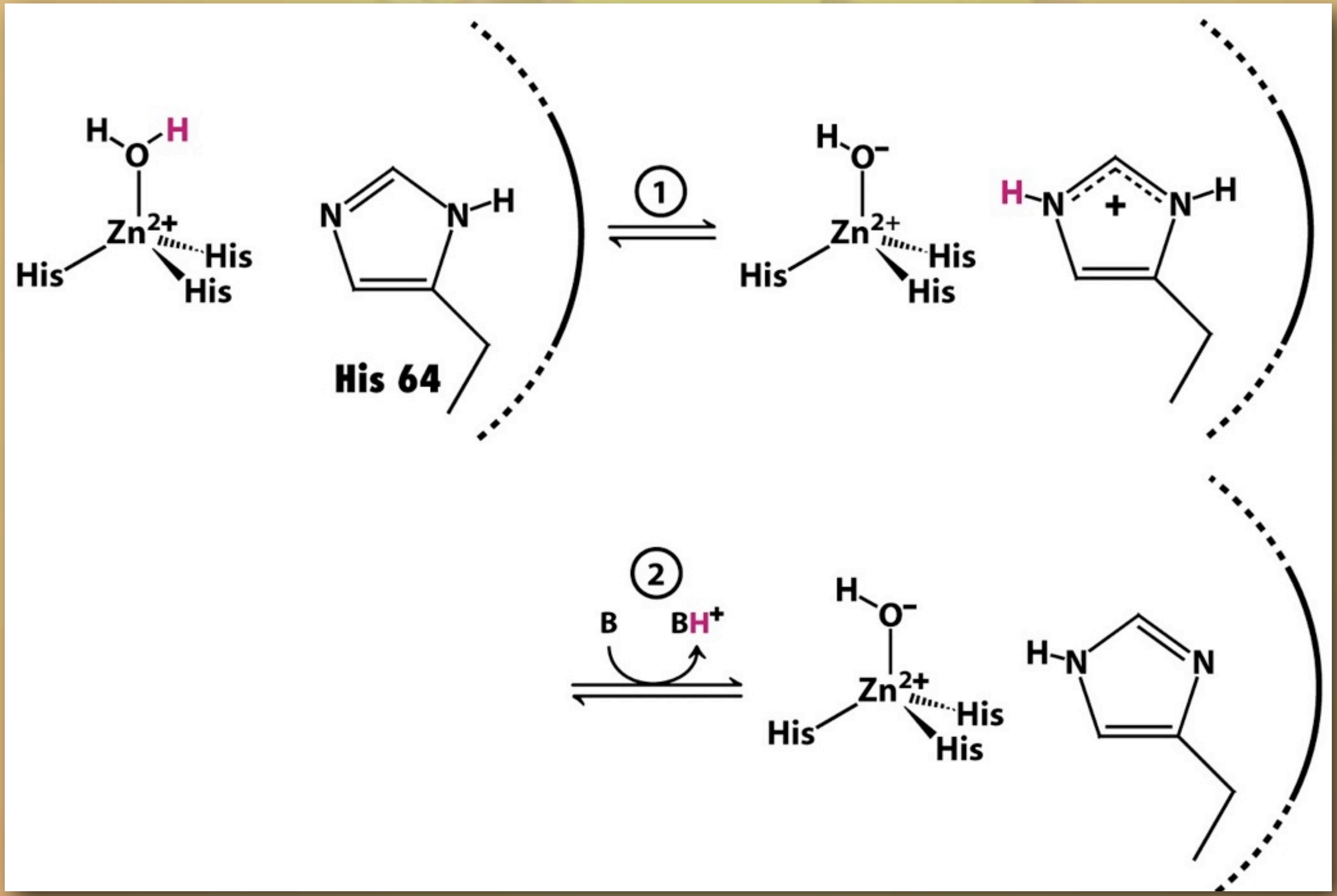
Carbonic Anhydrase

- Buffers provide a sink for the released hydrogen ions (H^+).



Carbonic Anhydrase

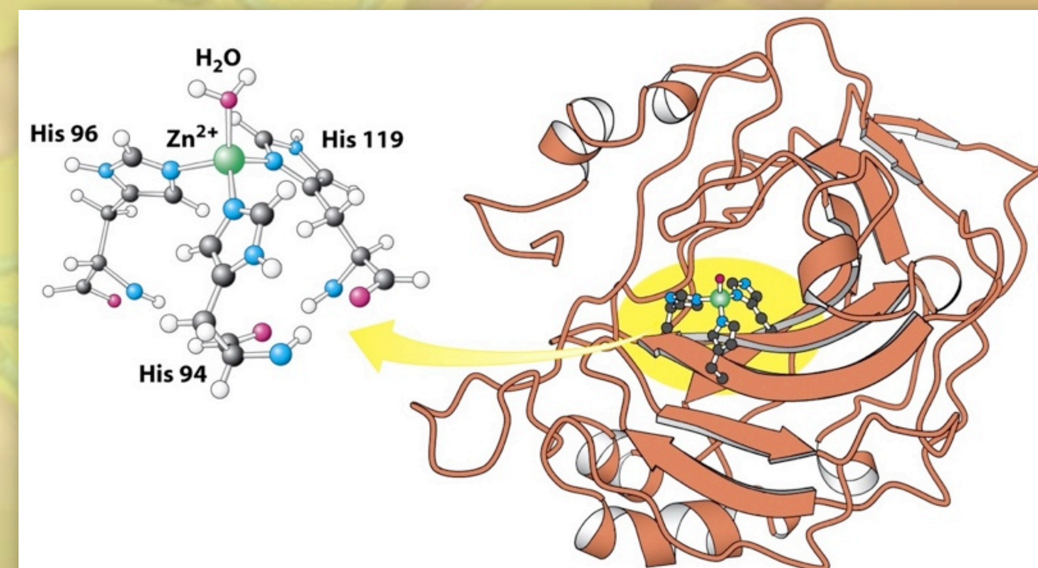
- His64 also helps mediate the the flow of H^+ away from the active site and to the buffer.



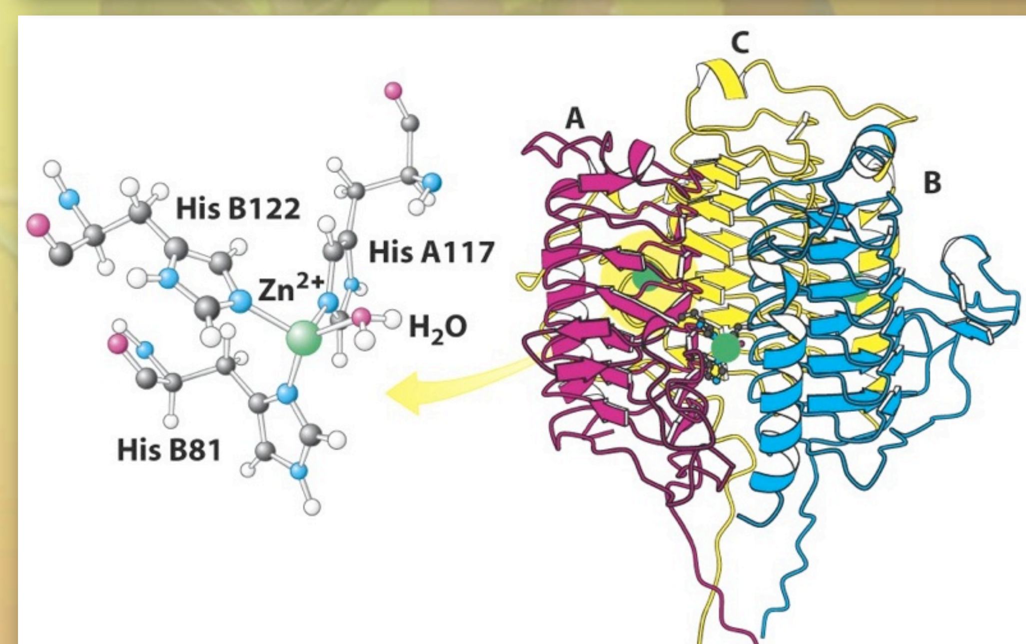
Carbonic Anhydrase

- Carbonic anhydrase also provides an example of **convergent evolution**.

α-carbonic anhydrase (Animals)



γ-carbonic anhydrase (Archean)



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EcoRV

- EcoRV is a **restriction endonuclease.**
  - It is a good model for demonstrating high substrate specificity.
  - The substrate is a specific sequence called the **cognate** sequence.

- EcoRV specifically cleaves DNA at the sequence **GATATC**
  - Like with many restriction endonucleases, the sequence for the complementary strand of the cognate sequence reads the same, but backwards.

<u>Restrictions sites share this common property</u>

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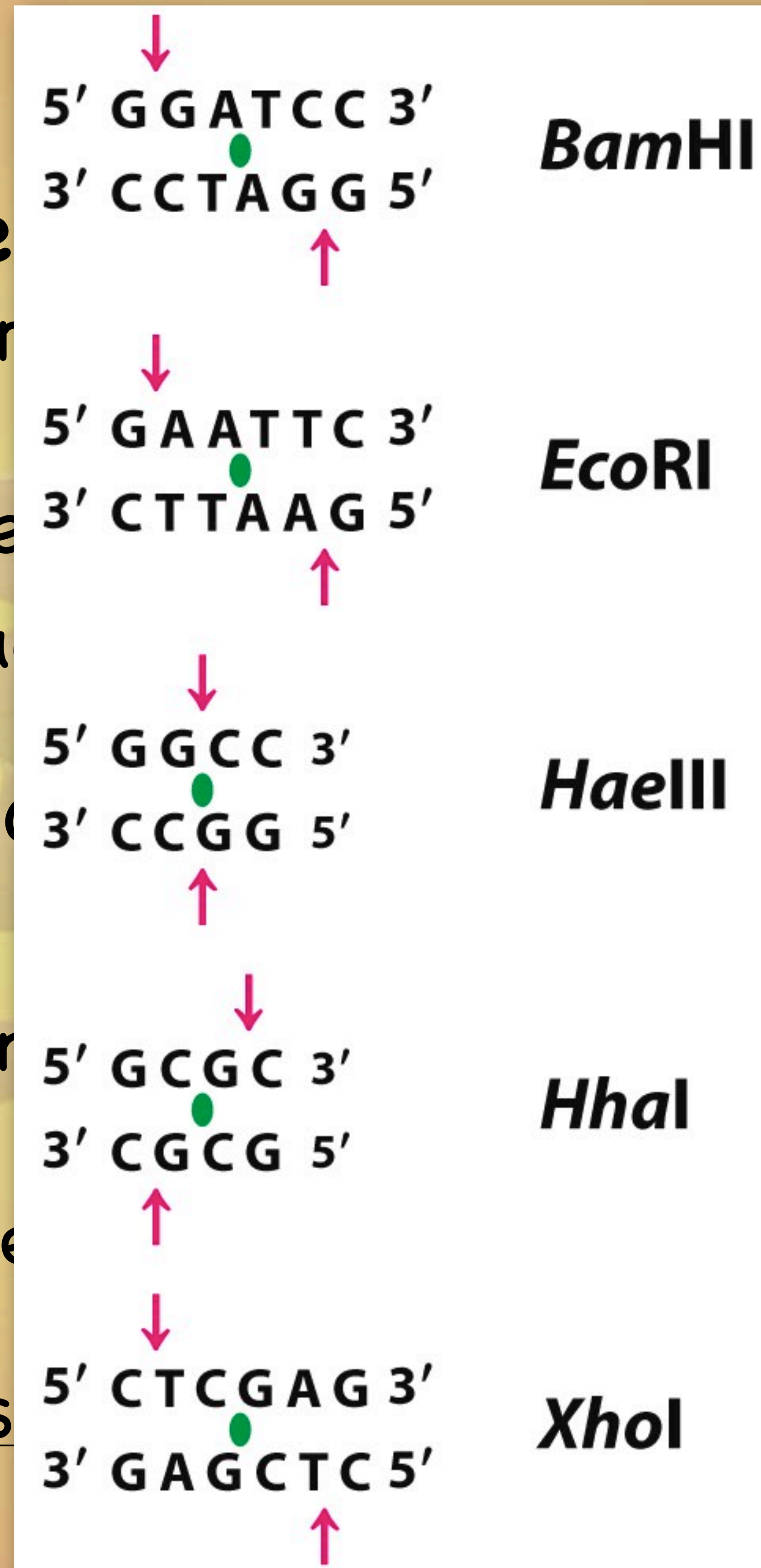
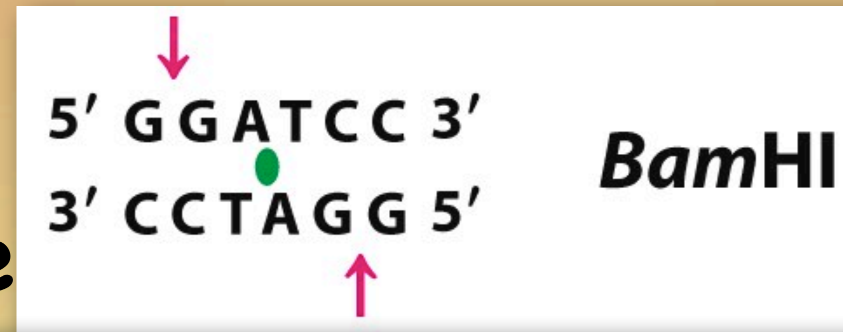
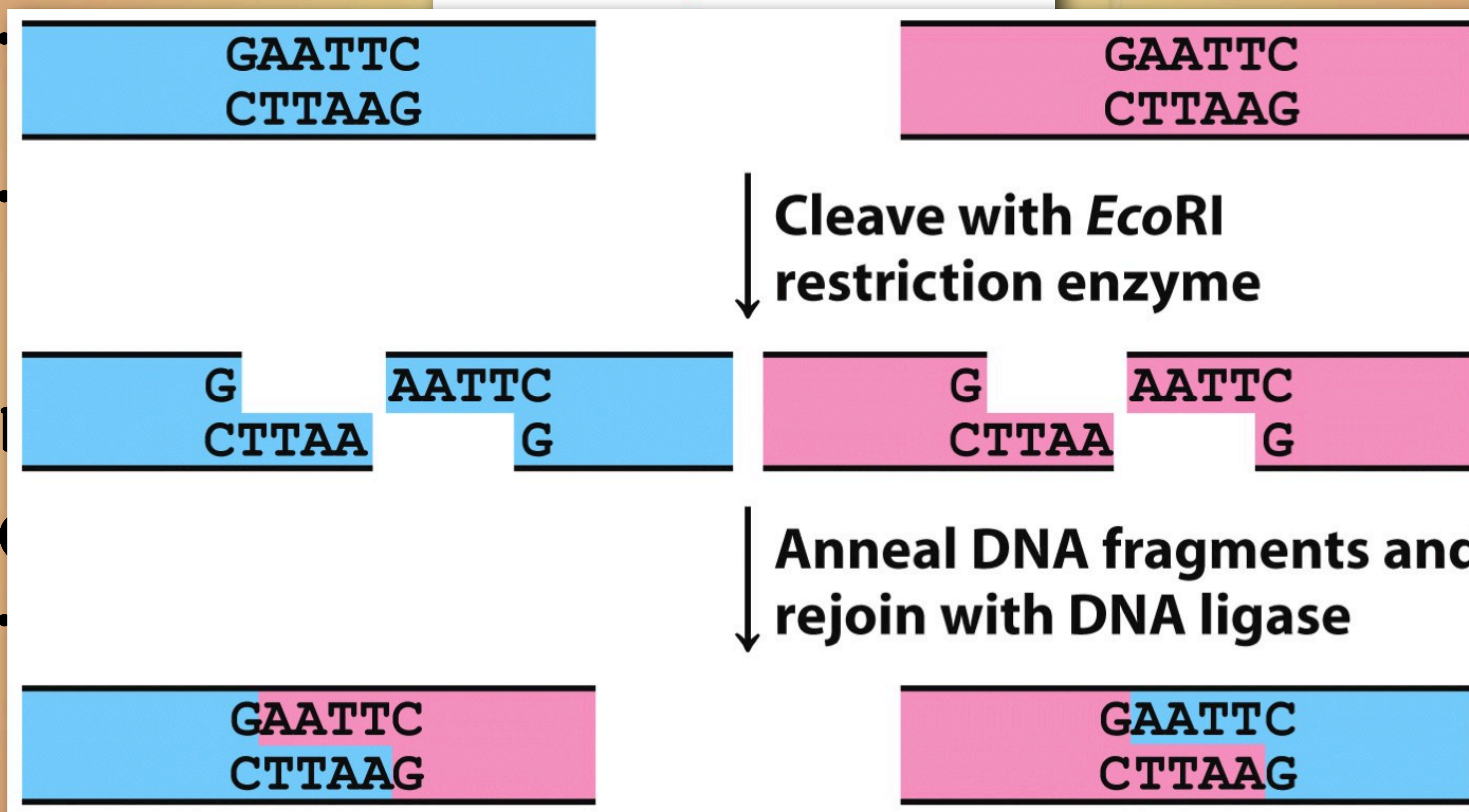
Restrictions on property

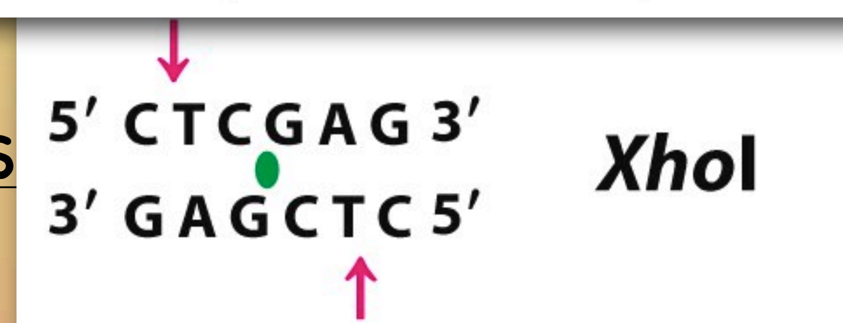


EcoRV

- EcoRV is a **re**... **ease.**
  - ...te
- ...ce







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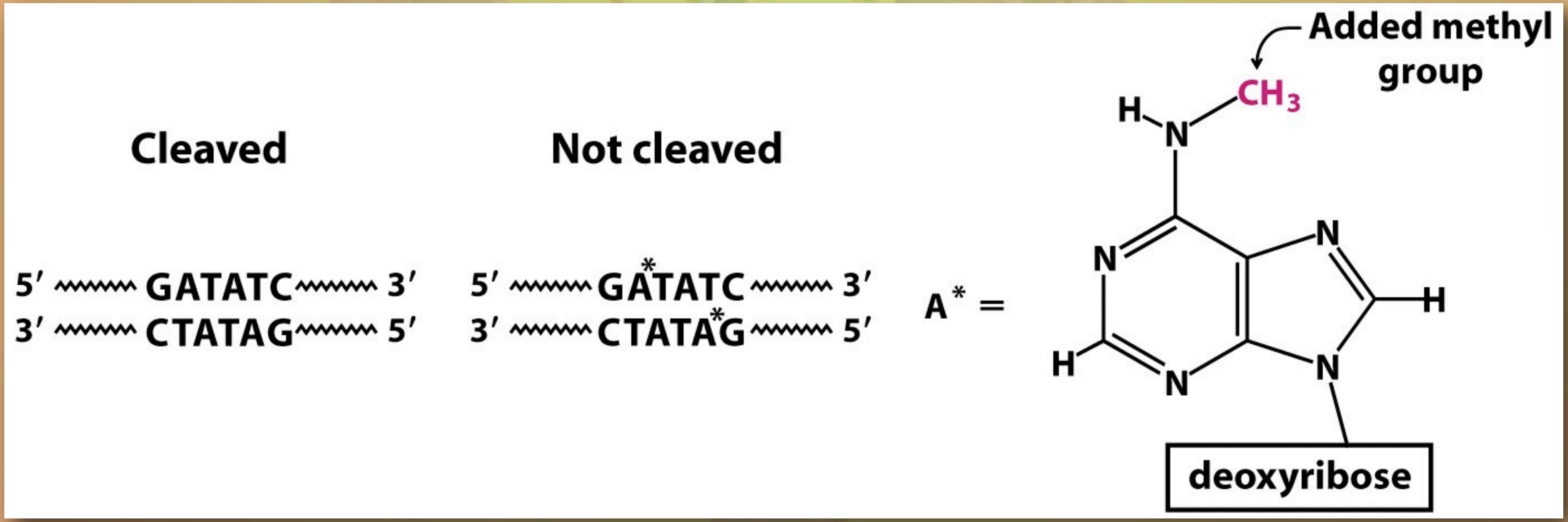
Restrictions sites share this common property

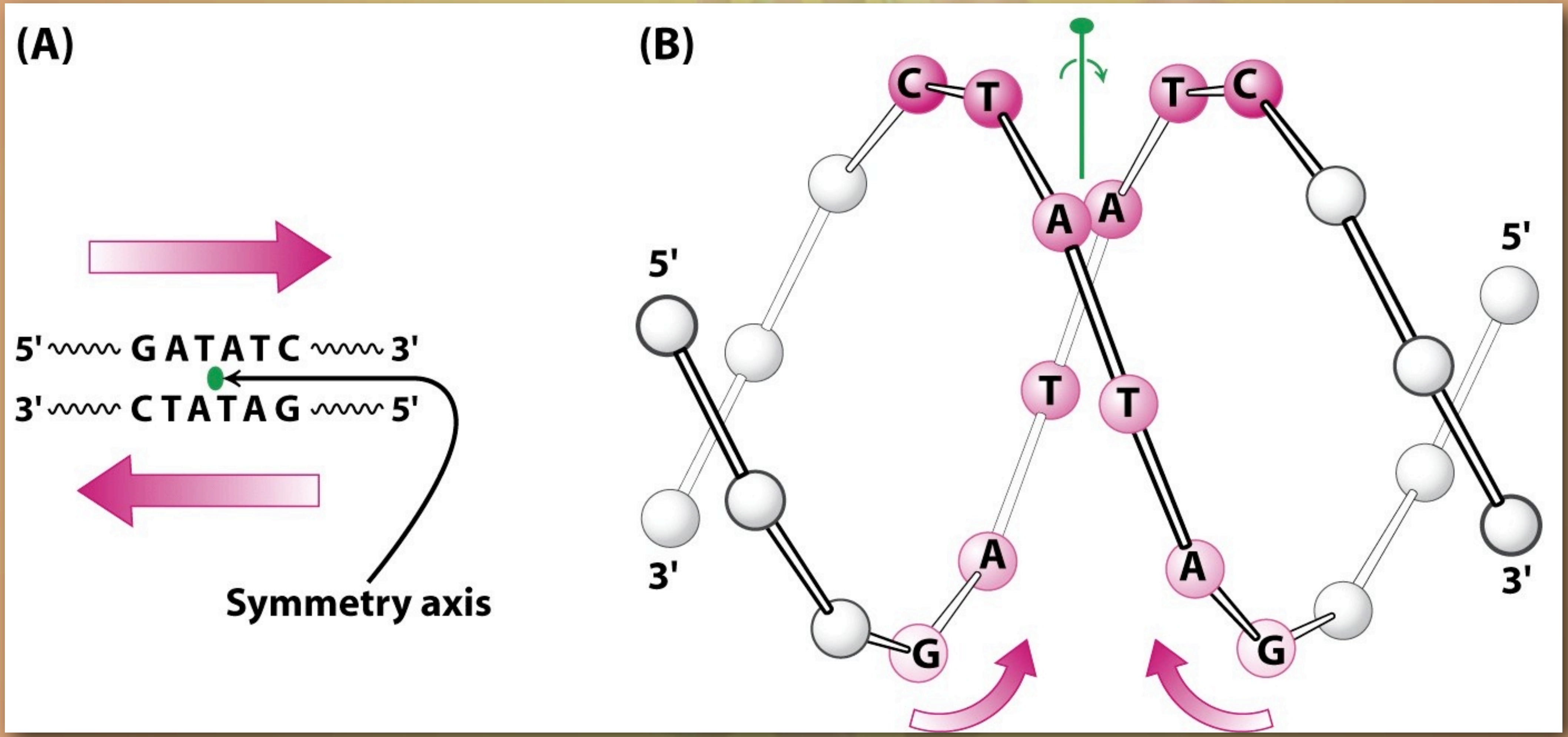
EcoRV

- EcoRV is a **restriction endonuclease.**
  - In conjunction with methylases, restriction endonucleases protect bacteria from viruses.
  - They have also become a powerful tool for molecular biologists.



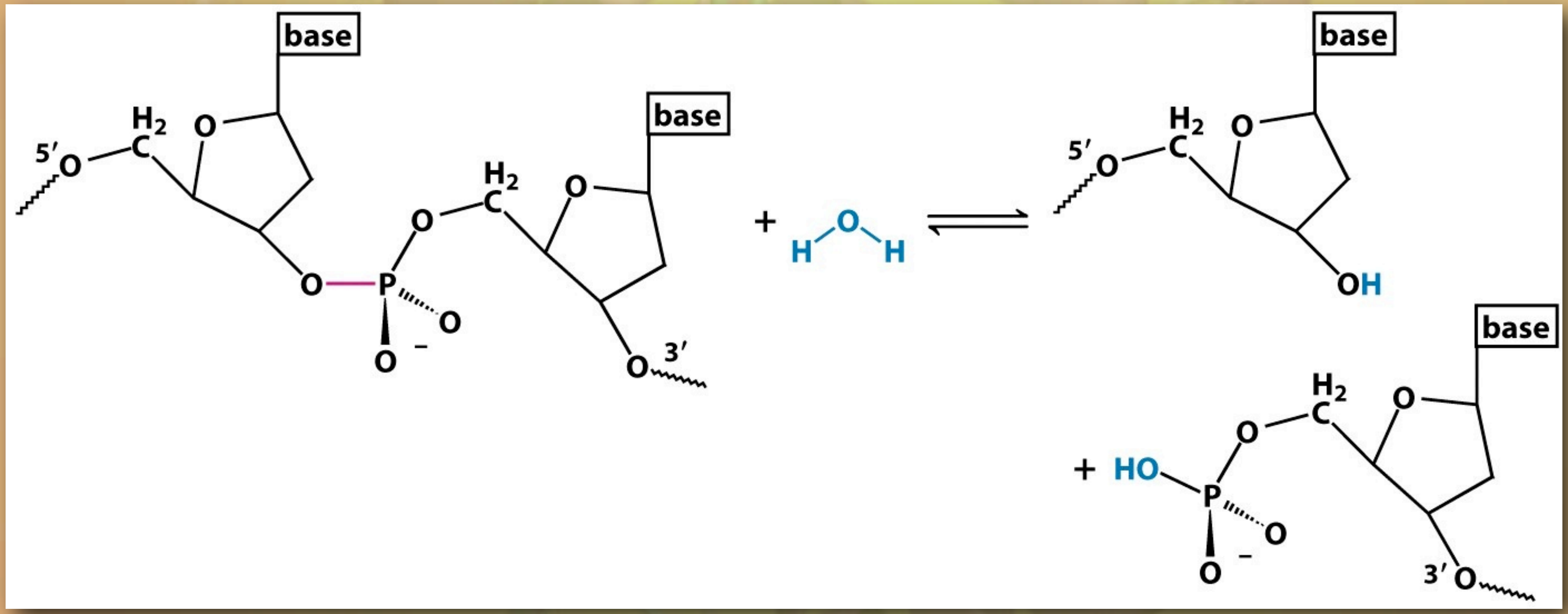
EcoRV

- Restriction sites have a 2-fold symmetry



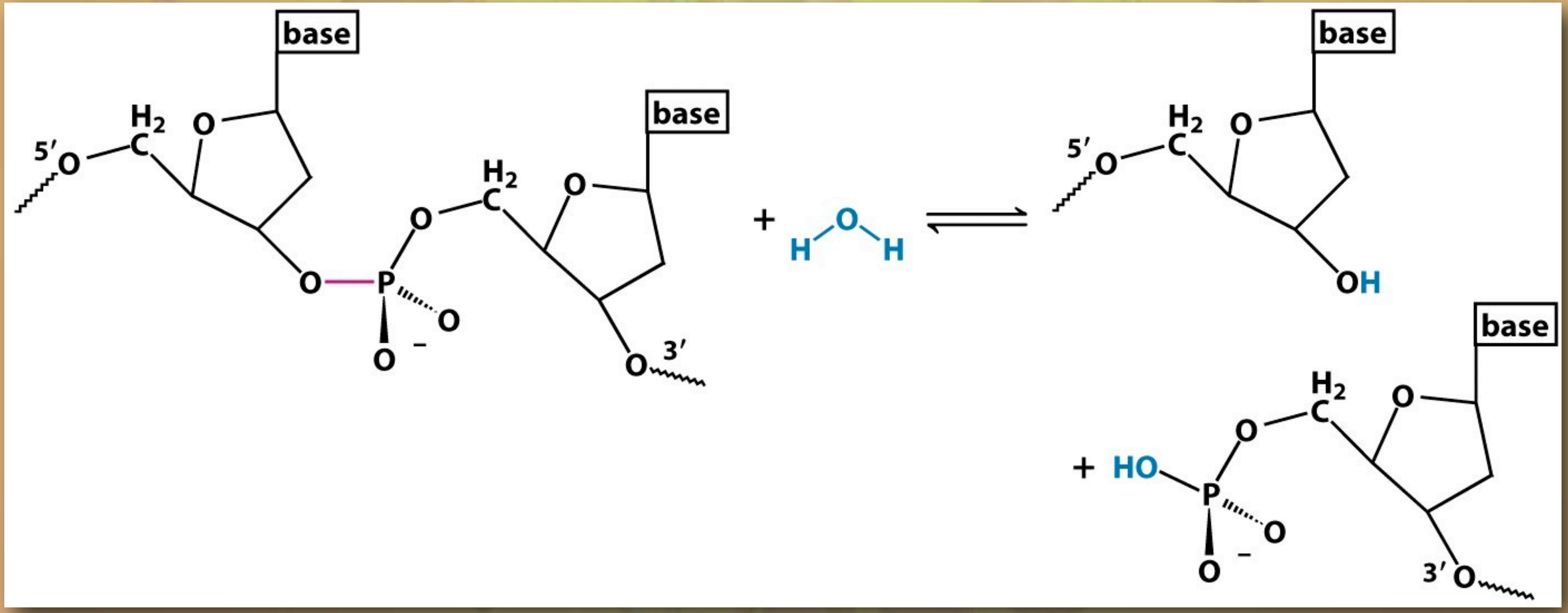
EcoRV

- Like chymotrypsin, the nuclease reaction is a hydrolase reaction.



EcoRV

- But does it also involve a covalently bound intermediate?



EcoRV

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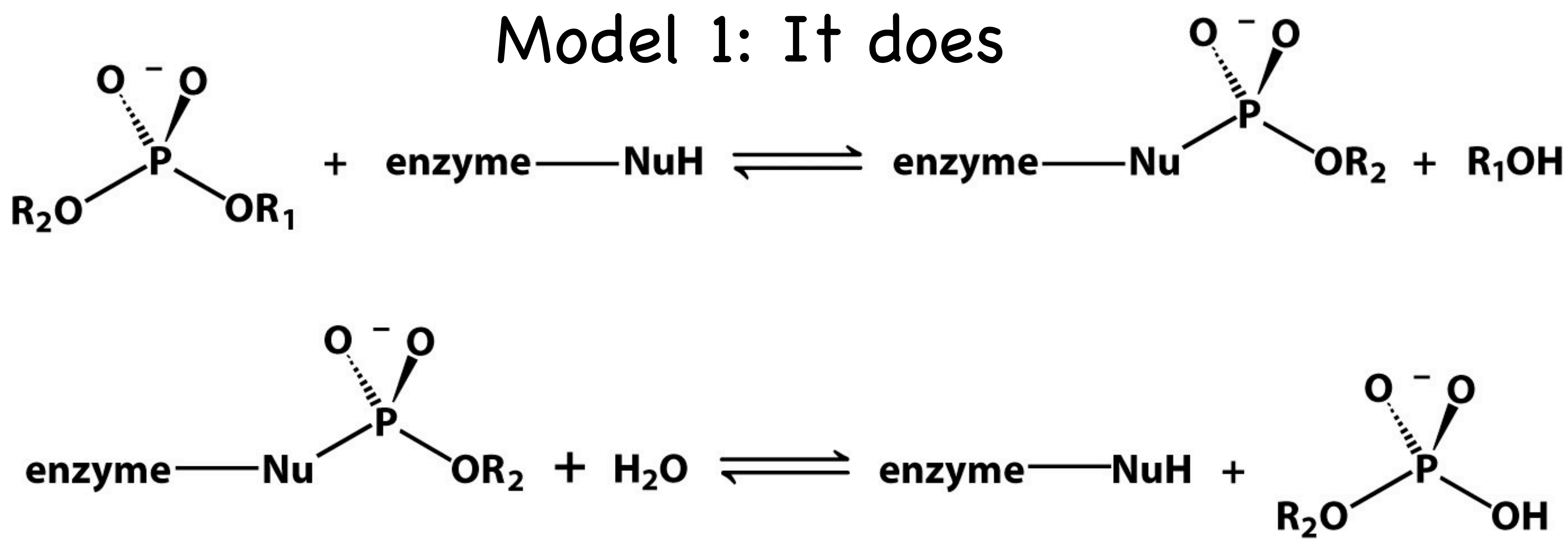
Model 1: It does



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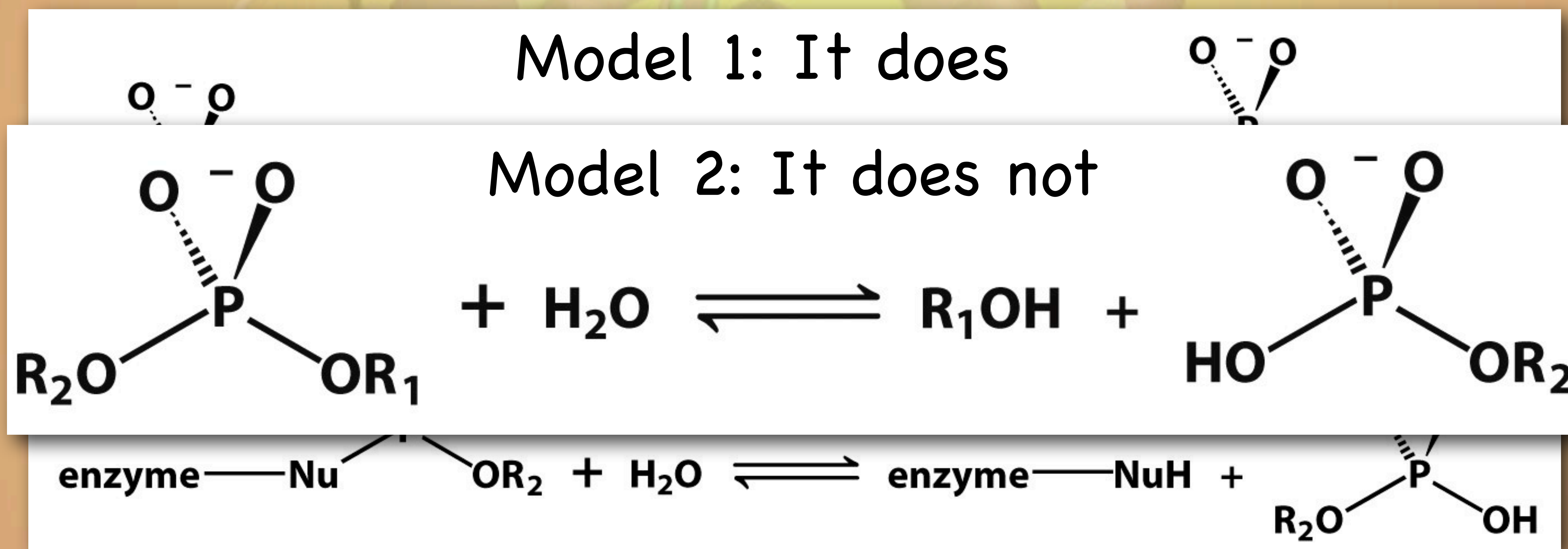

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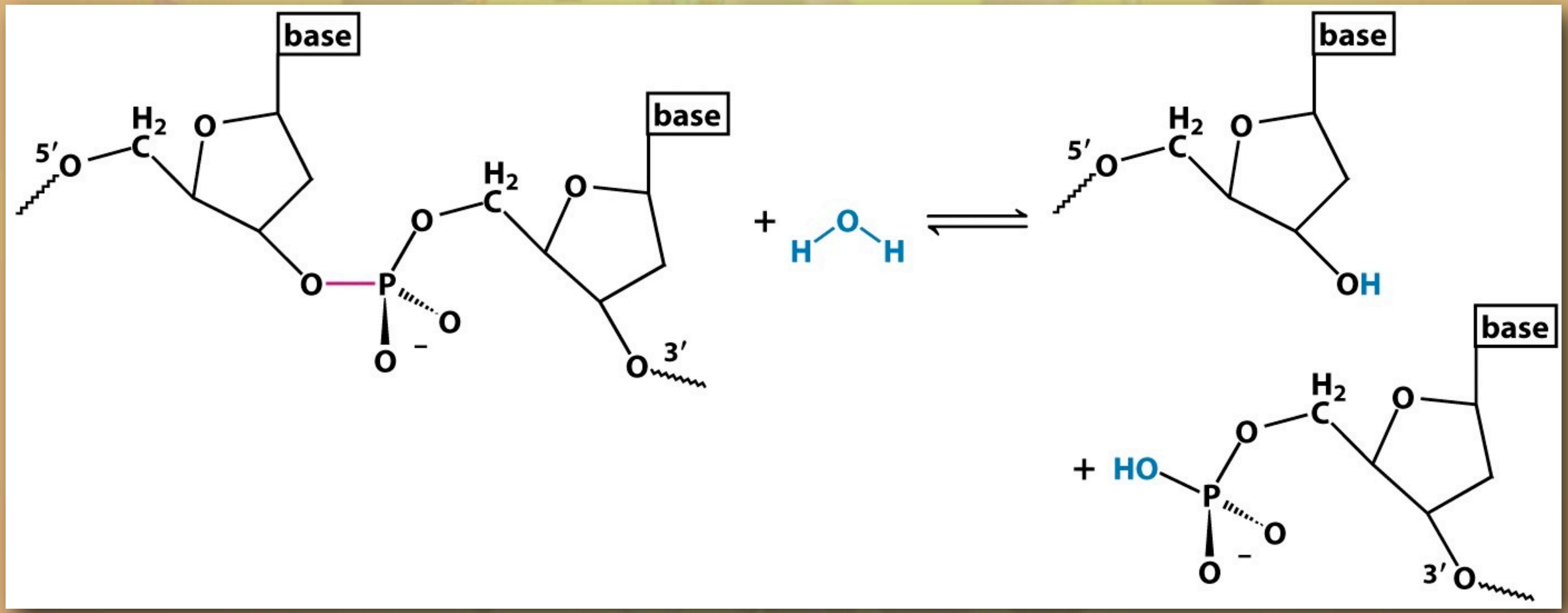
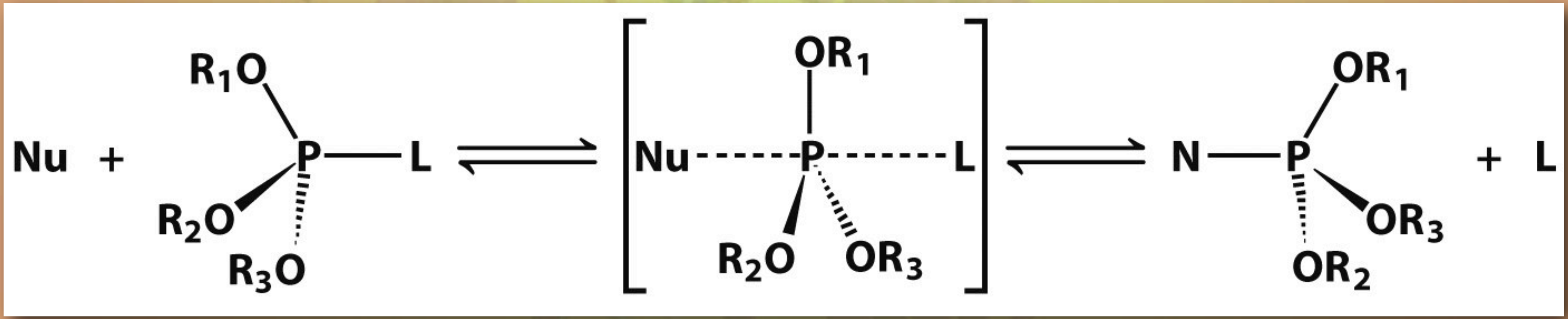


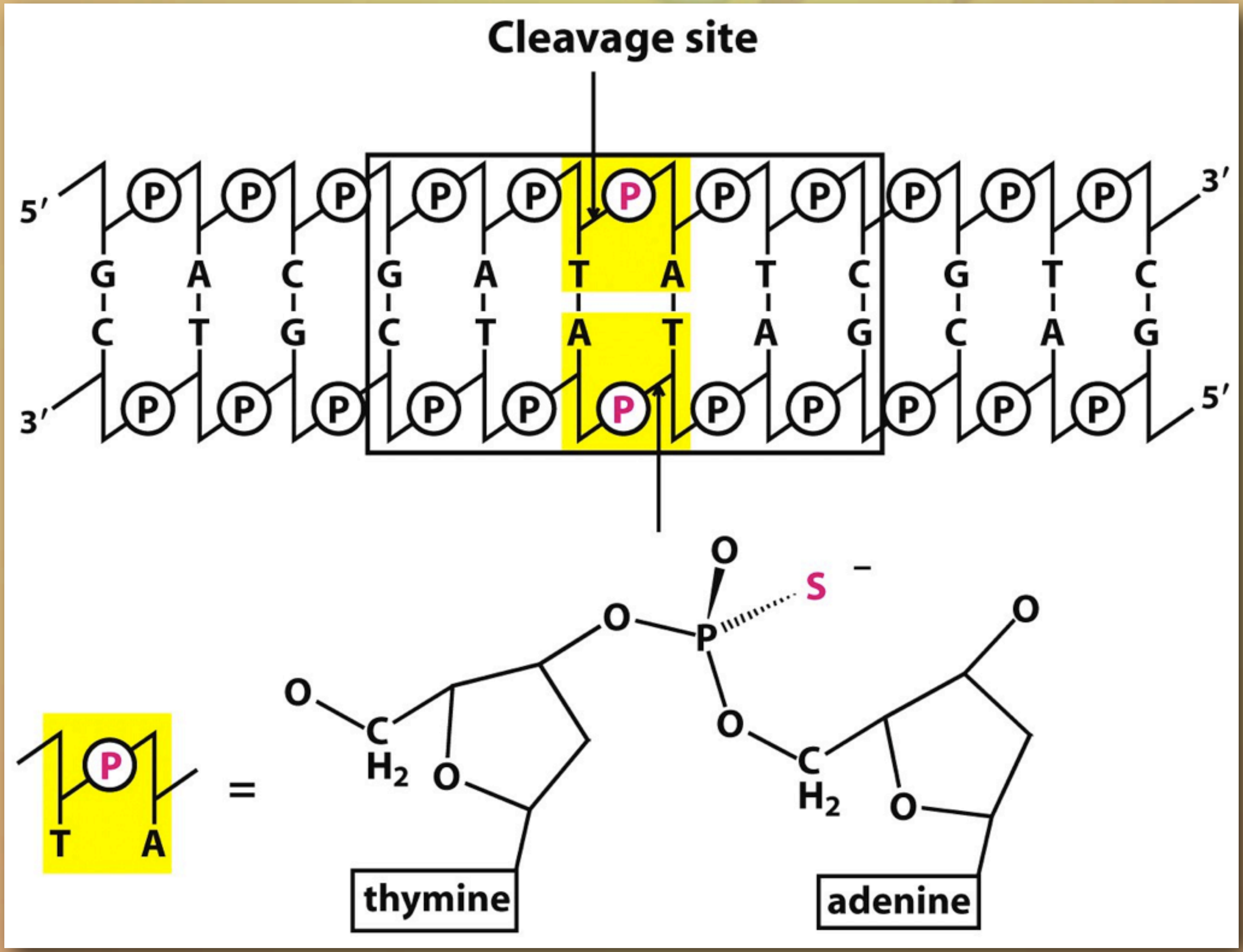
EcoRV

- Model 2 will invert the geometry about the phosphorous.
- Model 1 will not.



EcoRV

- A phosophorothionate label was used to answer this question.



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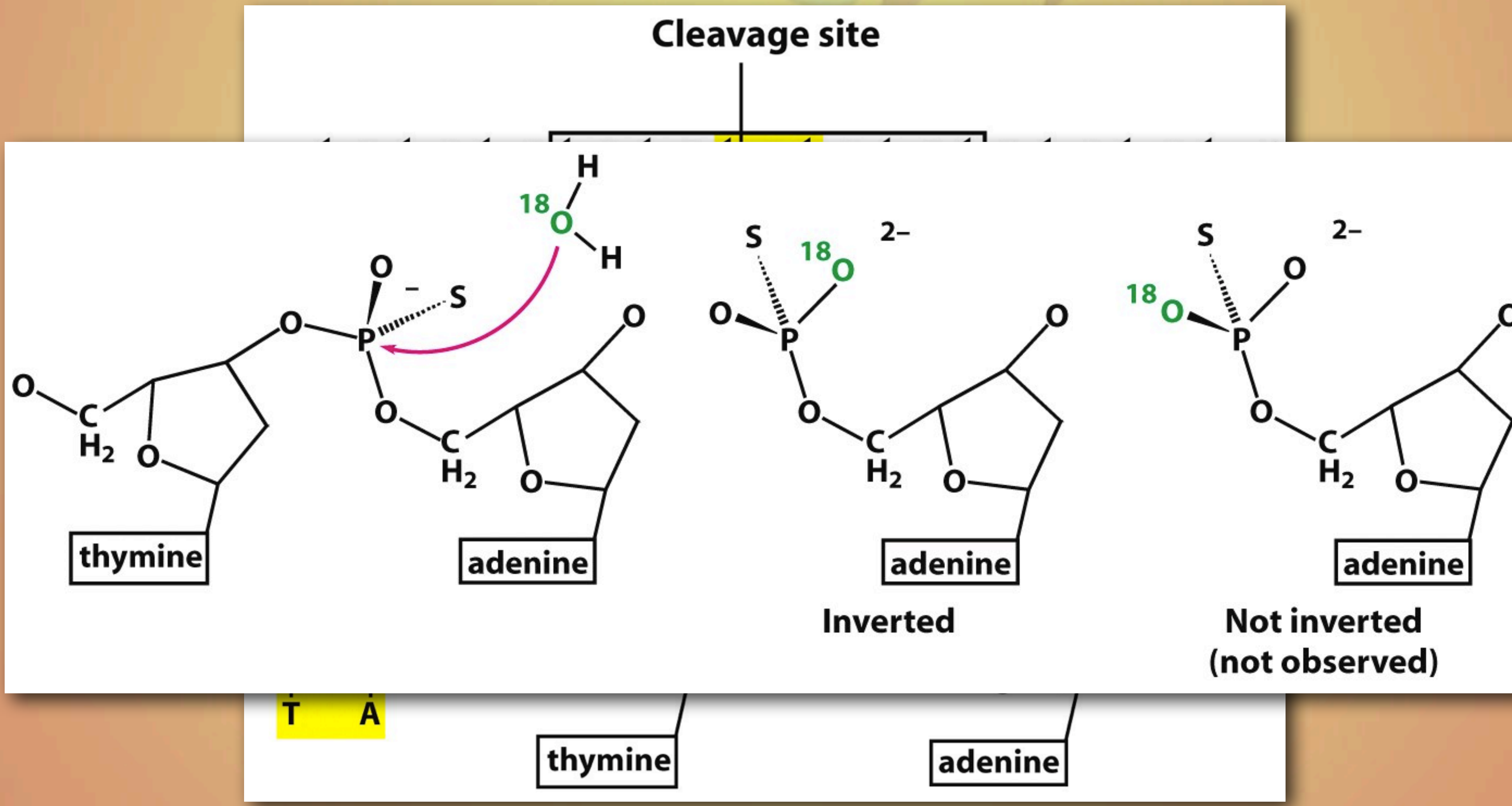
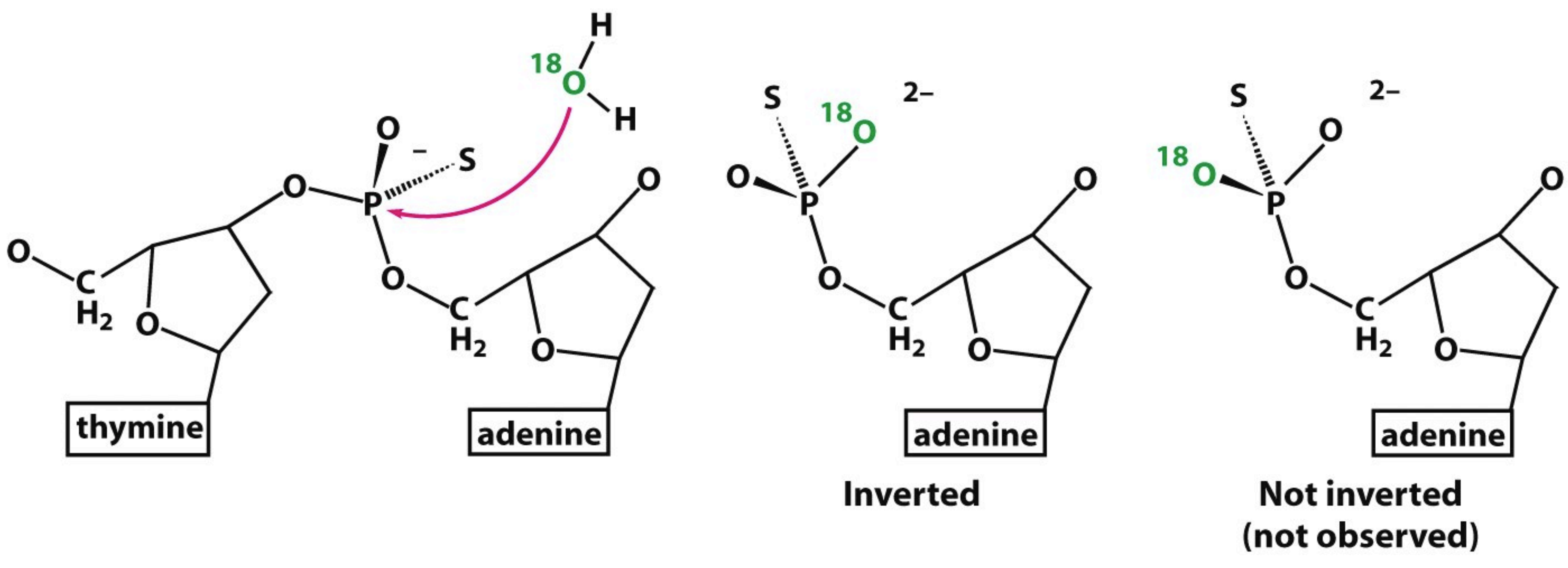


EcoRV

- And the answer is…
  - The symmetry is inverted
  - Therefore, Model 2 is the correct model.
    - The water reacts directly with the phosphate



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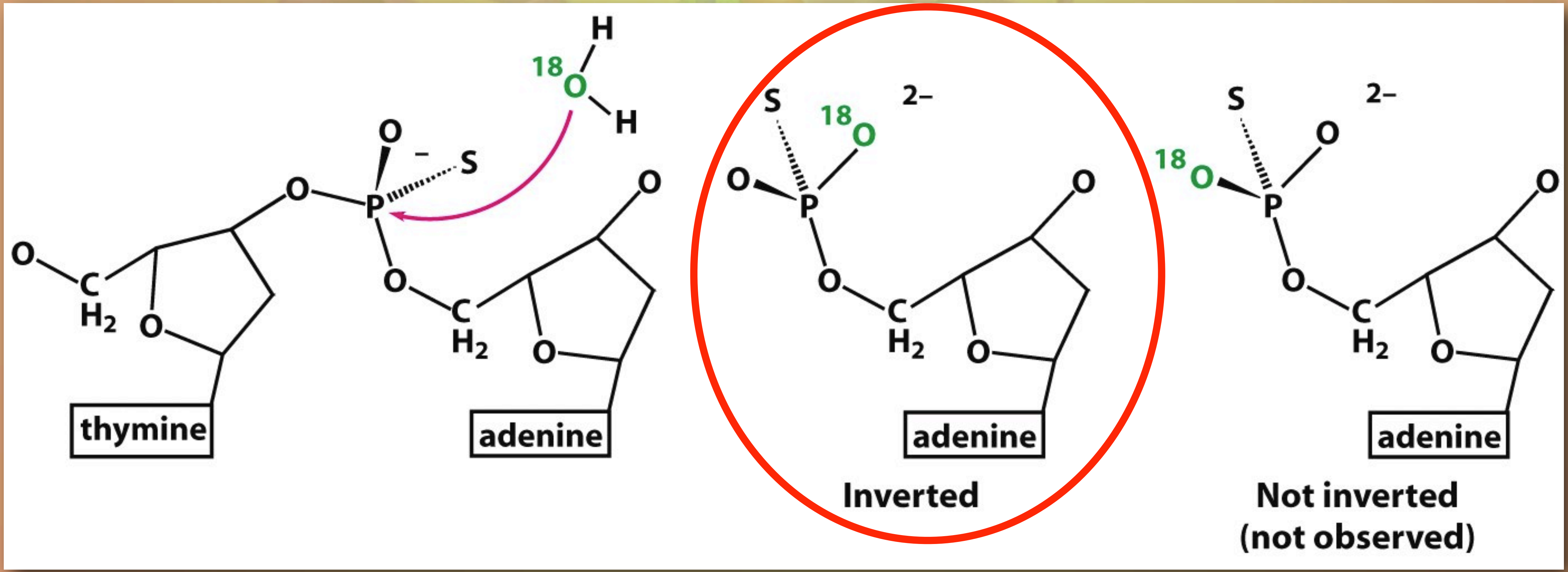

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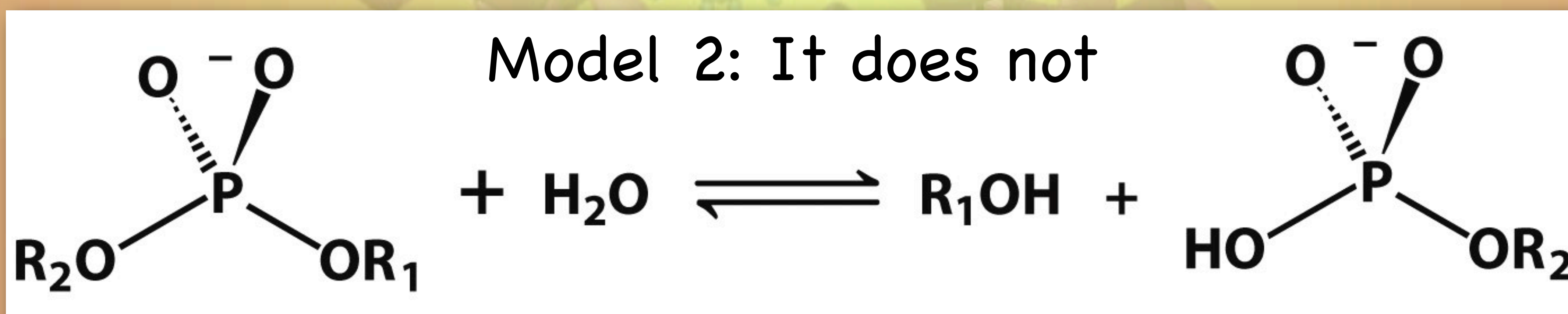
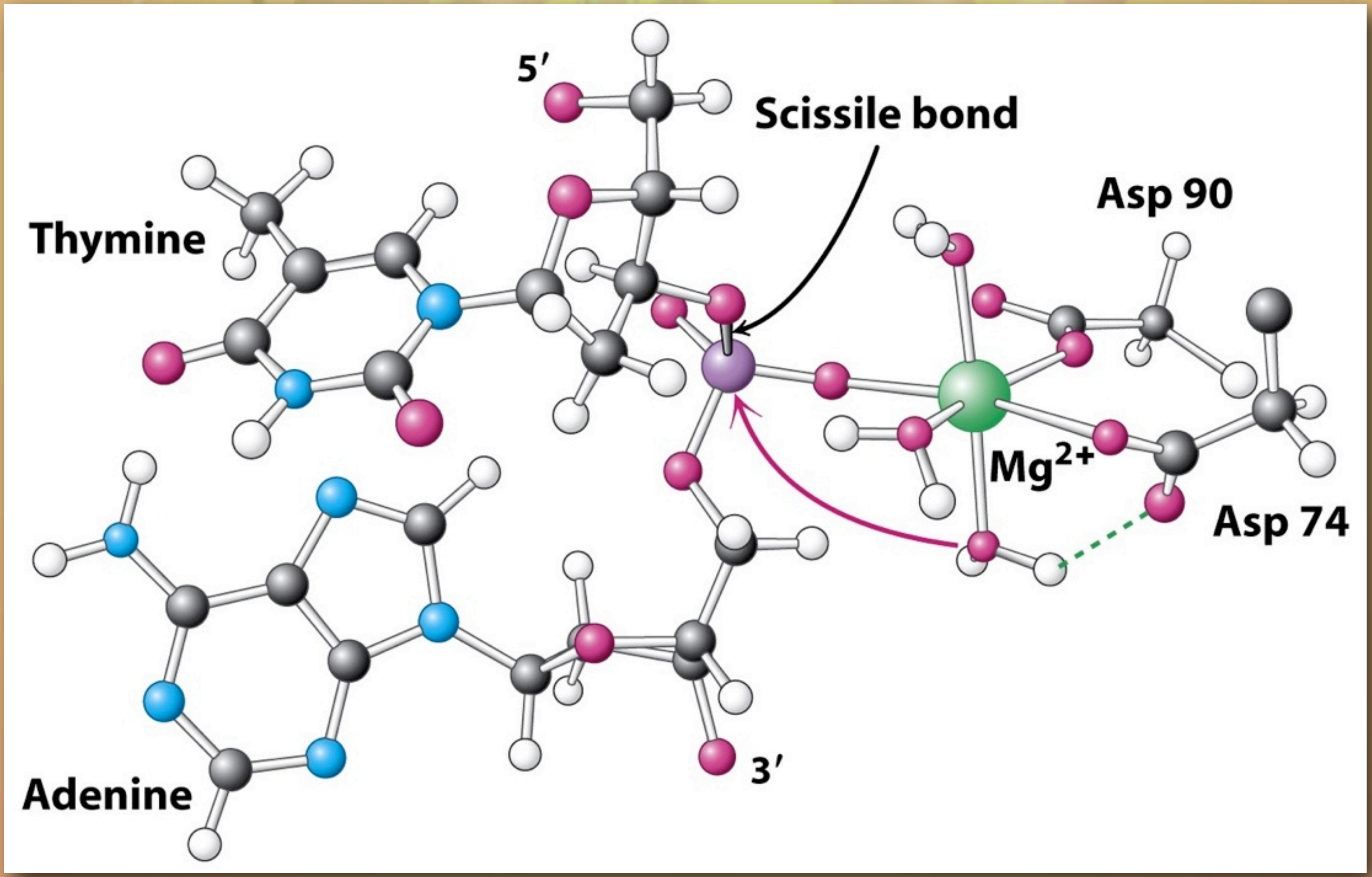


Model 2: It does not

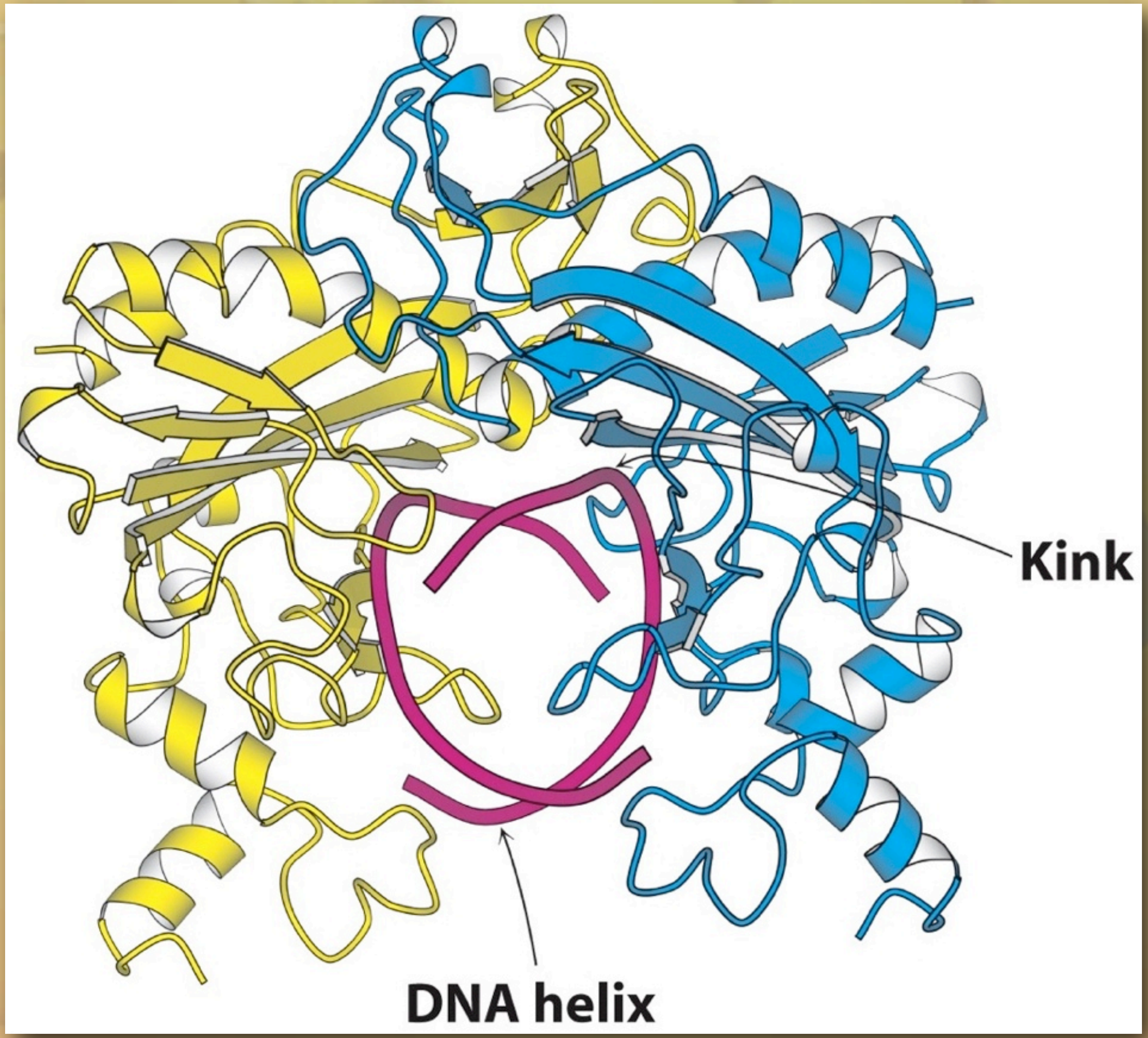
EcoRV

- Like carbonic anhydrase, a metal ion (Magnesium) is involved in generating the nucleophile.



EcoRV

- The active site is generated by a kinking of the DNA at the **cognate** site.



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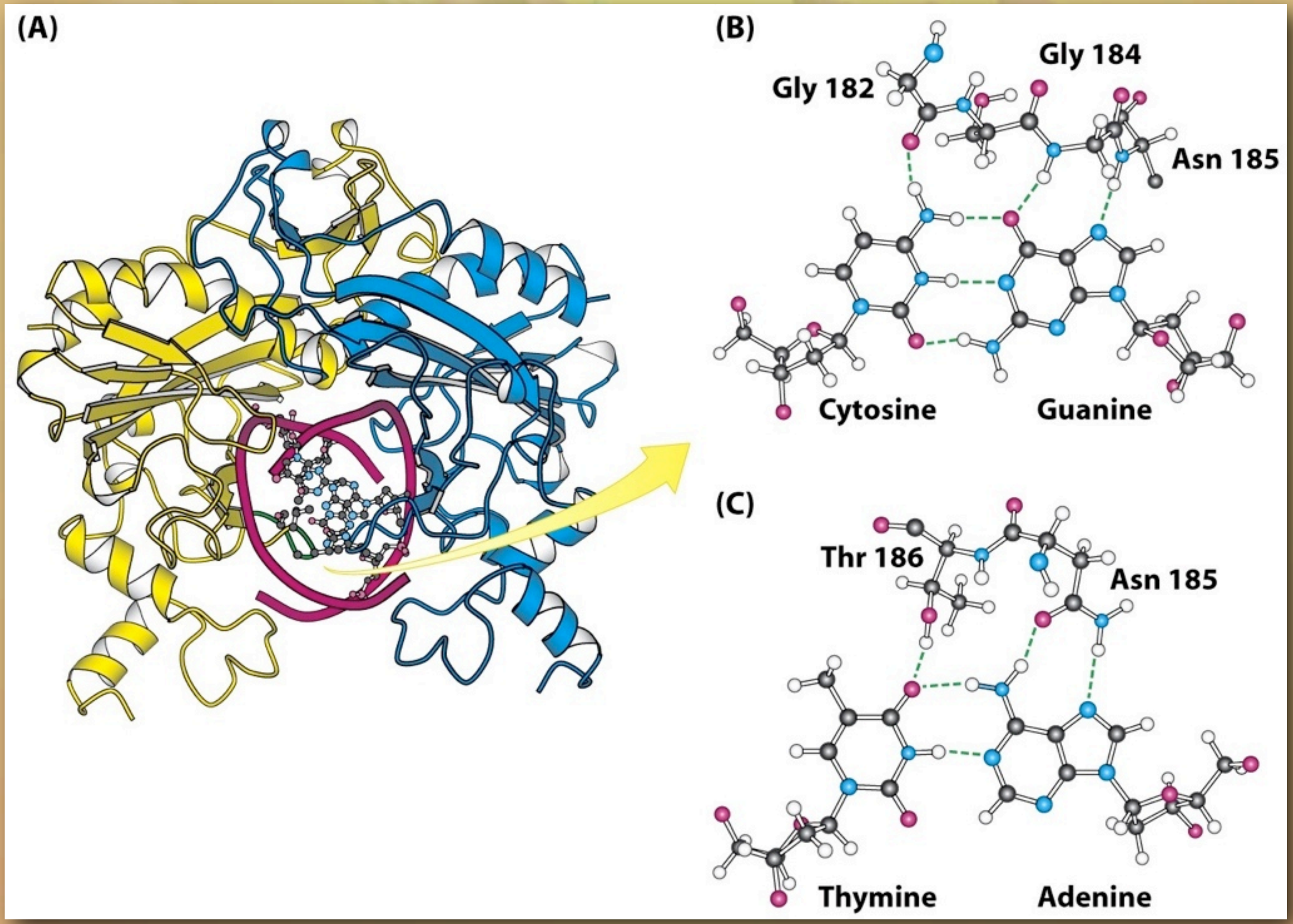

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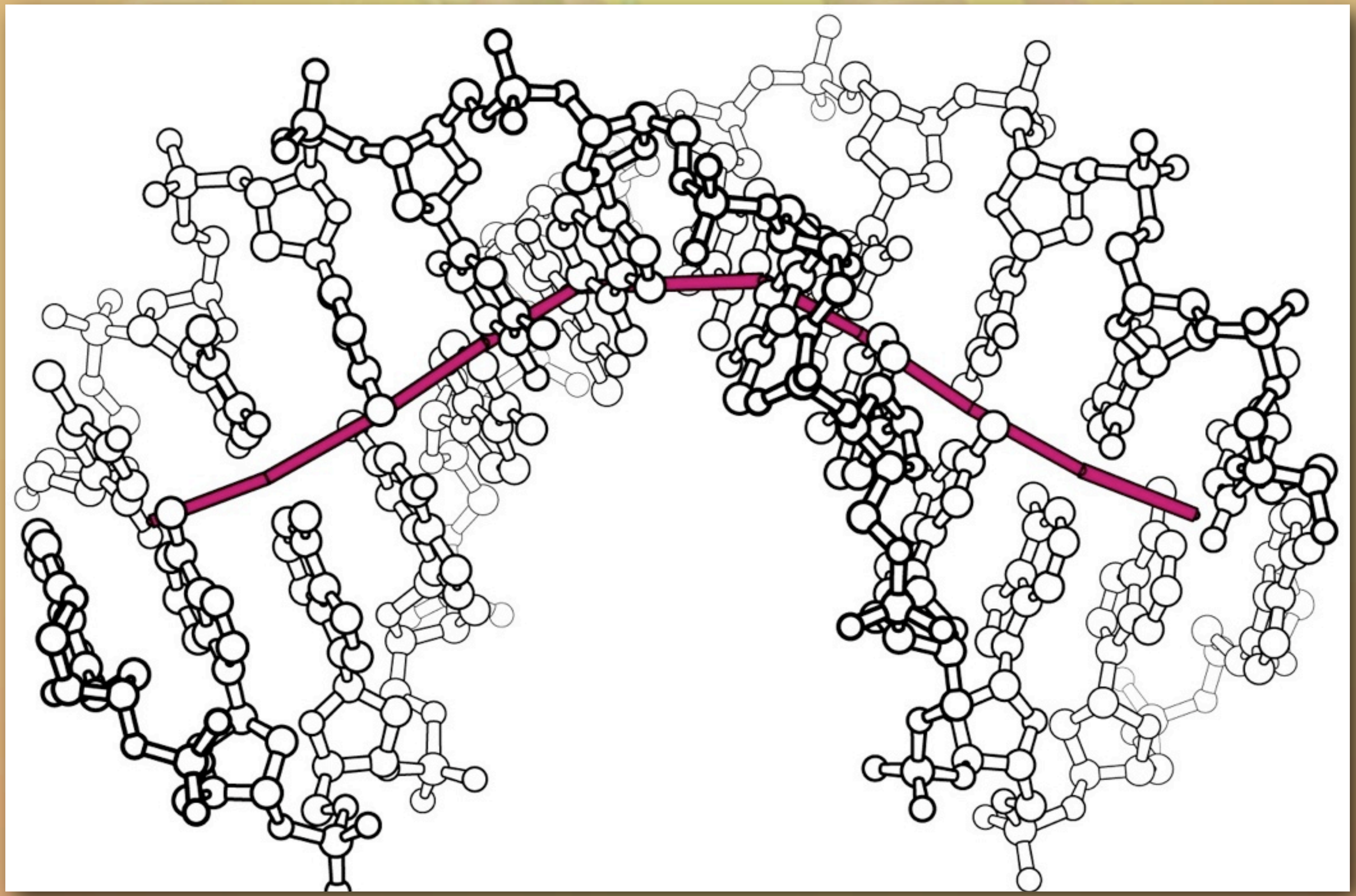

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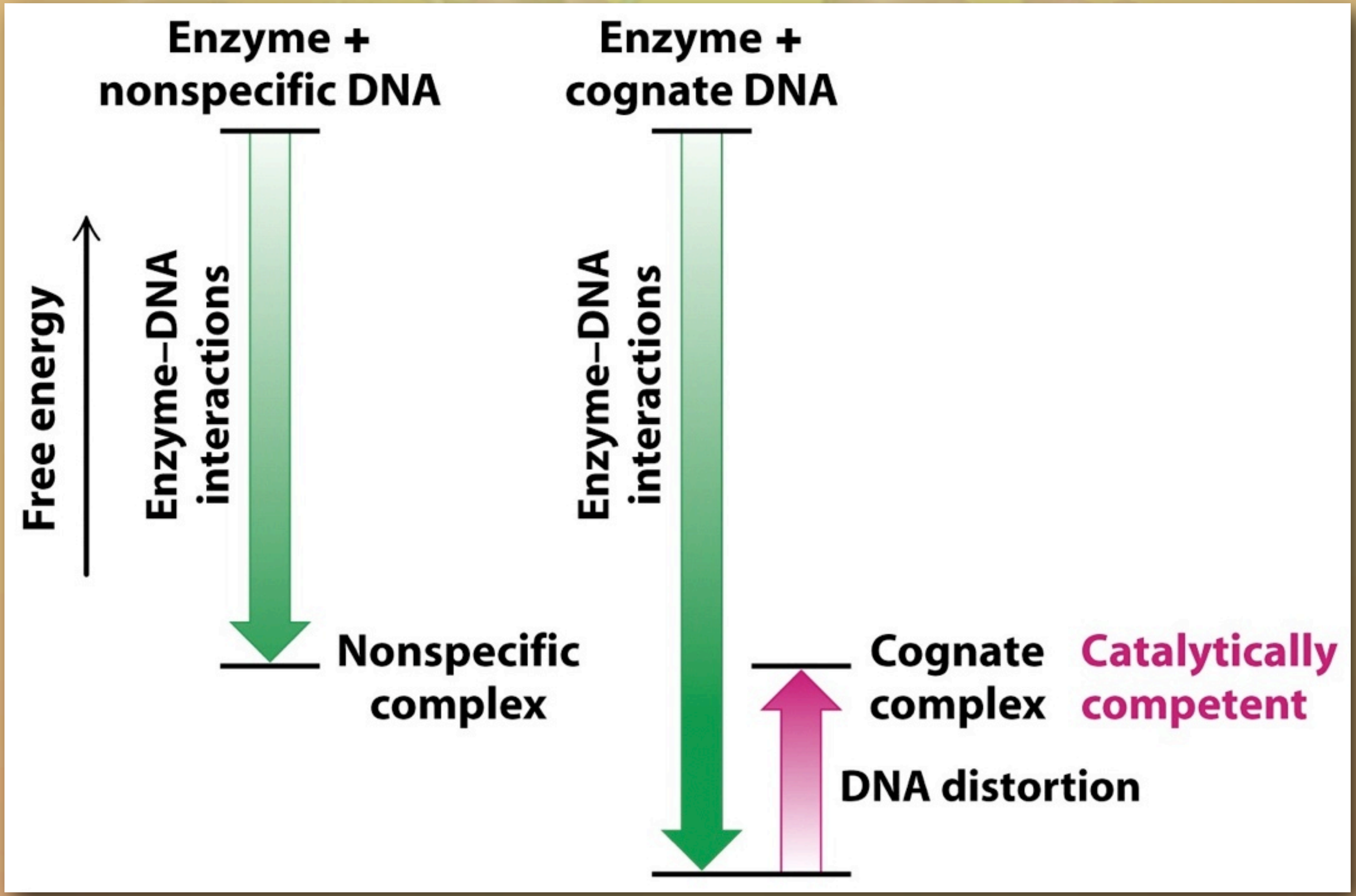

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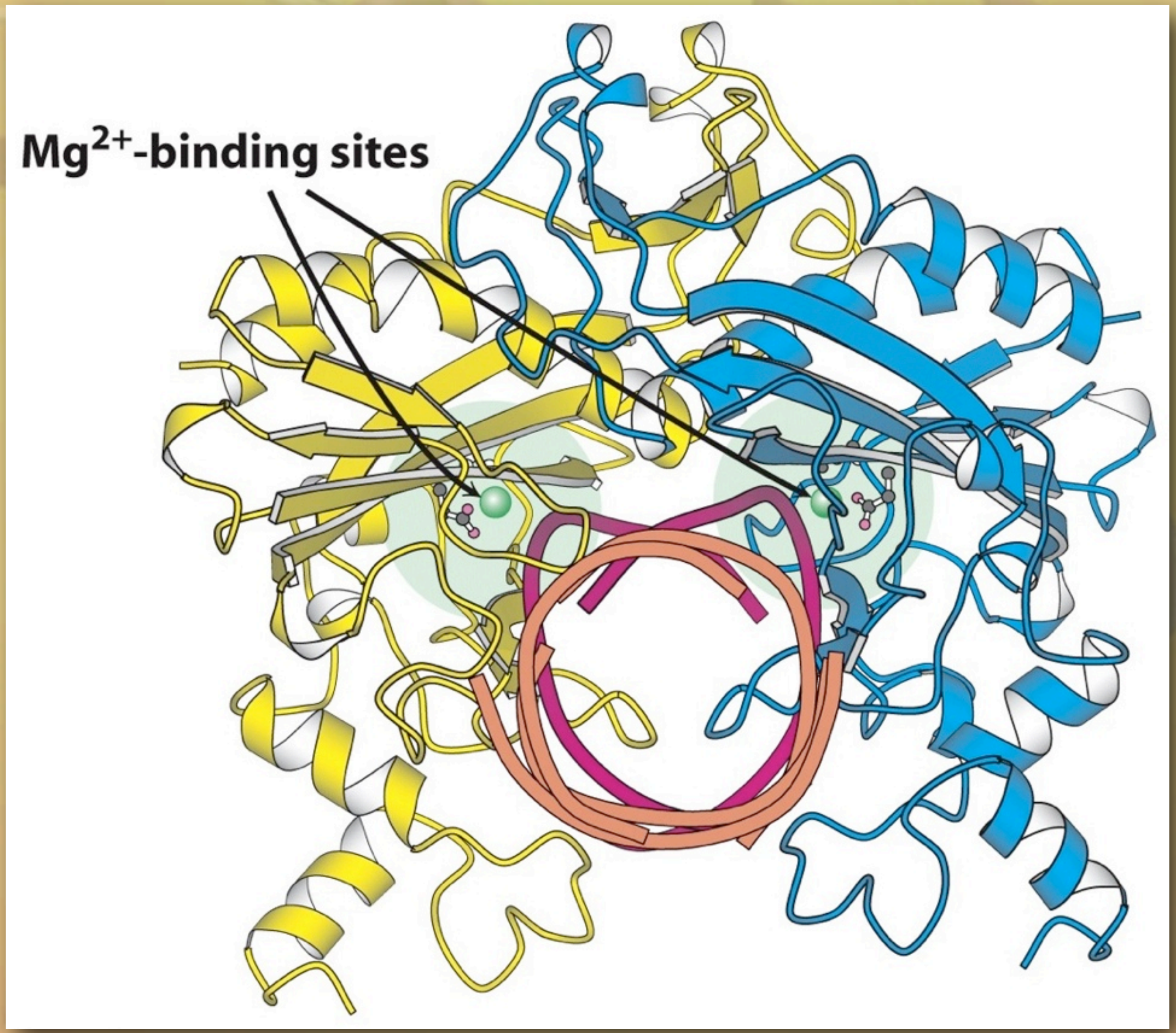

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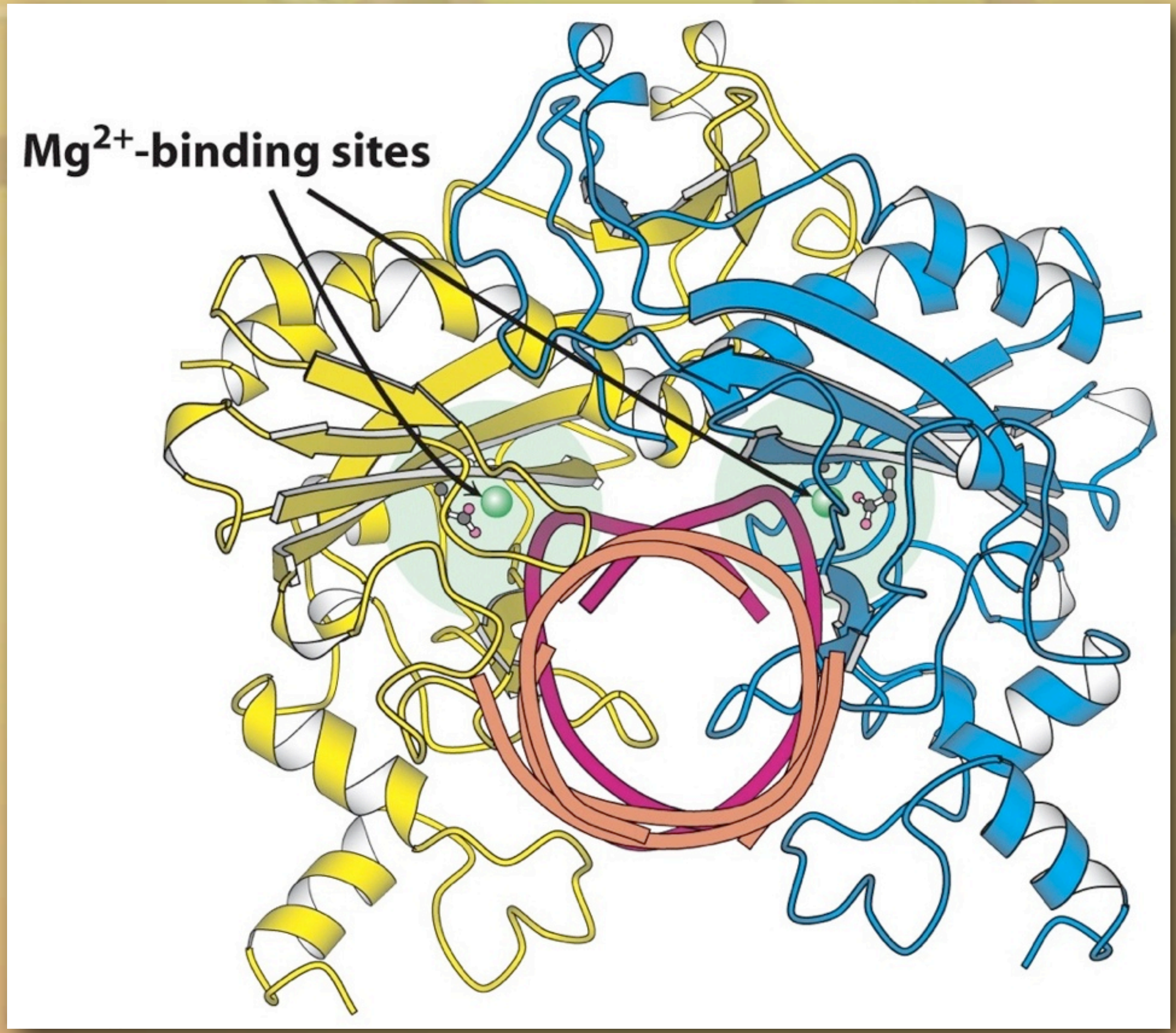
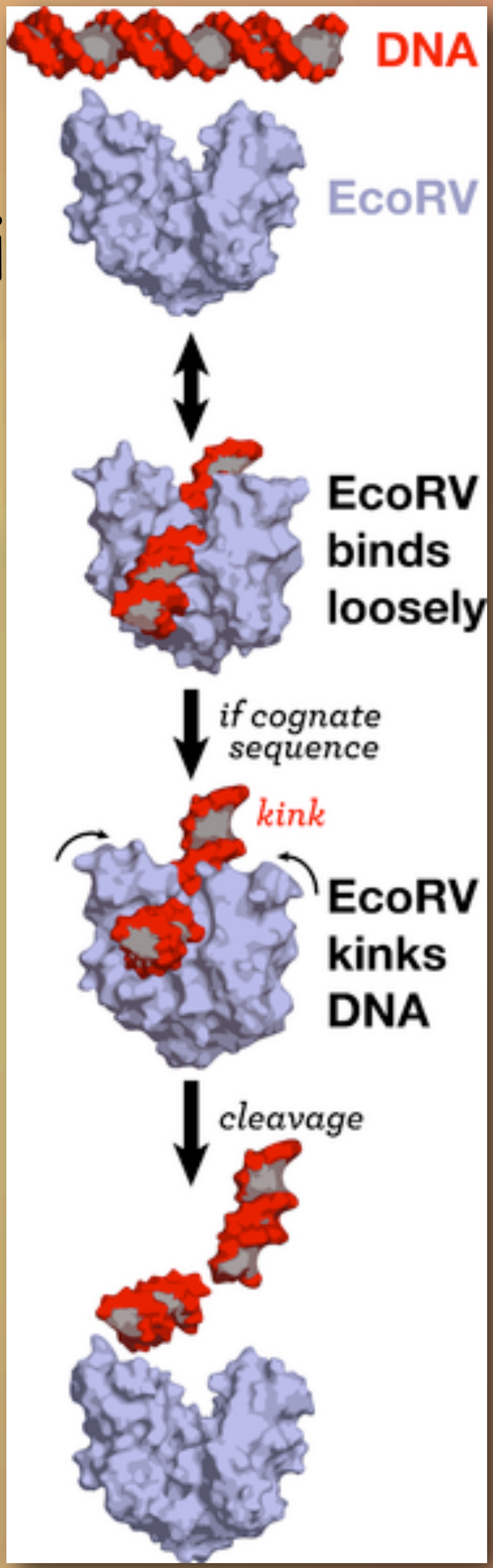
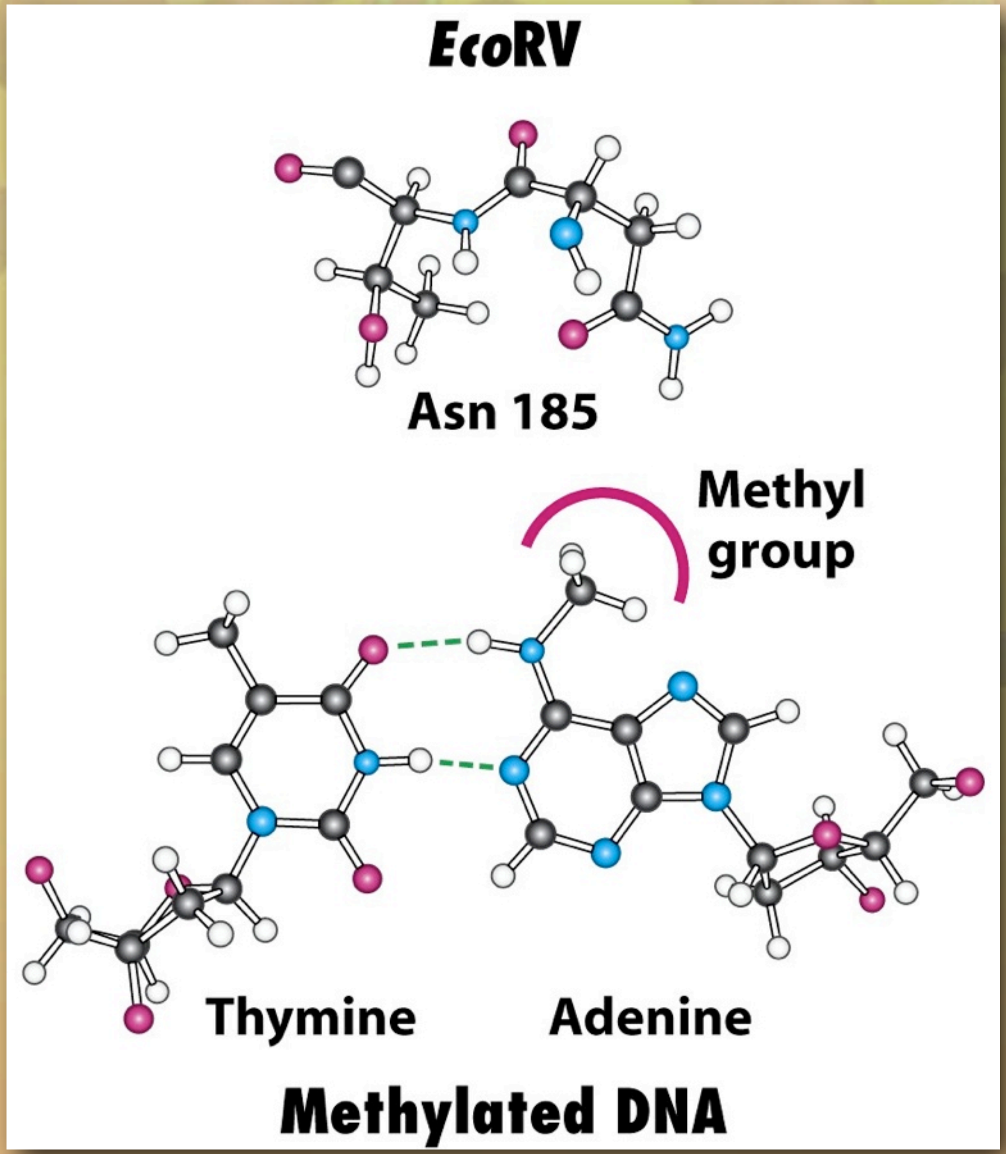




EcoRV

- The E. coli bacteria is protected from the EcoRV through a methylation tha blocks formation of the active site.



Next up

- Myosin motor domain ATPase (1fmv & 1fmw) 3.6.4.1 (Chapter 9)
  - An enzyme that couples the hydrolysis of ATP to the mechanical motion.
- Regulatory Strategies (Chapter 10)